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PAPER

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Influence of cold plasma treatment on insecticidal properties of wheat seeds against red flour beetles

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Abstract
For the long-term fortification of warehoused crops, the insecticidal impact of cold plasma treated wheat seeds against red flour beetles are studied. In this study, the gain weight loss, mortality and repellency tests of cold plasma treated seeds are investigated and statistical analysis is carried out by SPSS software. Cold plasma treatment is carried out at 800 V for 1 and 4 min. The results show that the minimum grain weight loss is observed in plasma treated seeds as compared to controlled seeds. It is also observed that the mortality rate is minimum in the controlled seed while maximum at 4 min plasma treatment. Similarly, the repellent effect has a very small variation as compared to the controlled seed for *Tribolium Castaneum*. Therefore, plasma treatment can be considered as a practical and effective method for seed protection against red floor beetles particularly during long-term storage by controlling the insecticidal effects.

Keywords: cold plasma, mortality, repellency, grain weight loss, quality, stored seeds protection

1. Introduction

Tribolium Castaneum (Herbst) is an international beetle pest of warehoused crops and can invade a variety of products. A number of efforts have been reported to find substitutes to control the residue-free non-toxic pest [1, 2], one such novel method is a cold plasma (CP) treatment and rf heating [3–10]. CP or non-thermal plasma treatment is a comparatively advanced and emerging technique for microbiological decontamination and sterilization of food [11–14]. CP was used to investigate the input variables as a function of power, treatment time and distance between electrodes. It was concluded that the effect of low and high pressure plasma could be a promising nonchemical and nonthermal method for integrated pest management in stored food products [15].

CP has the ability to sterilize and kill bacteria, yeast and other hazardous constituents of seeds and food supplements [16–19]. The CP treatment can be used to improve the germination, promote the growth and increase the physiological level of wheat, leading to an enhanced yield [11, 20].

Plasma treatment requires no chemical input or genetic manipulation to the seeds and is a uniform and less damaging method. CP treatment has a good effect on disease resistant abilities [21]. Plasma treatment along with radio wave technologies substituted the traditional pre-sowing and post sowing strategies for chemical seed treatments which may have hazardous effects on human health and environment. This treatment leads to the suppression of plant pathogens, such as fungi and bacteria, as it helps to reduce such infestations [22]. Mihai *et al* [23] investigated the germination rate, and growth rate of radish seeds before and after plasma treatment. They found the increase in root and sprout length, which was about 11% and 10%, respectively. They also reported an increase in the root weight which was almost 15% higher than the root to shoot mass ratio. They reported that this plasma treatment had a significant effect on the germination rate for the time interval of 420 min. Many authors [9, 14, 20, 21] reported that contamination of seeds with fungi can be processed in plasma at low temperatures which have a positive effect. Therefore, the pesticidal effects of plasma treatment against red flour beetles are important to study. The objective of the present study is to investigate the effect of CP on the insecticidal mortality, repellency and weight loss against wheat seeds on red flour beetles. The results obtained from the plasma processing of these wheat seeds were very encouraging for the storage of these seeds for a longer period of time.

2. Materials and methods

In this project, wheat seed samples named Galaxy-2014 were obtained from the Ayoub Agriculture Research Institute (AARI), Faisalabad, Pakistan for CP treatment. Among 79 wheat seed varieties released by AARI, Galaxy-2014 is one of new varieties discovered on 2013 that has been cultivated in Punjab (Pakistan). A sowing period of this variety is from 10th November to 15th December and it is cultivated in all irrigated areas of Punjab, Pakistan.

3. Plasma treatment to wheat seeds

Low temperature CP treatment was done at Quench Age Industry, Sialkot, Pakistan. This reactor has a gas chamber with two electrodes of variable potentials. The optimum pressure was kept fixed to avoid the chances of swelling, color changing of seeds or contamination from the atmosphere. Argon gas was introduced as feed gas in the chamber. As the required potential was applied the argon plasma was generated and then the seeds were subjected for plasma processing. Initially the seeds were treated for different treatment time of 1–8 min for different applied voltages of 200–800 V. Finally, the plasma treatment was performed at applied voltage of 800 V for time interval of 4 and 1 min. The statistical analysis has been performed by using ANOVA to study the weight loss and molarity at different plasma treatments of seeds.

3.1. Tribolium culture

A *Tribolium* culture was taken from wheat flour. This initial culture was incubated at 27 °C, the beetles were then then released in controlled and plasma treated seeds. The ideal temperature for red floor beetles is 27 °C–40 °C; below 18 °C, the beetles do not exist.

3.2. Grain weight loss test

Weight loss of plasma treated wheat seeds was performed by Padin *et al* [24] with minor modifications. Treated wheat seeds were taken and then damaged as *Tribolium* was a secondary pest. These treated wheat seeds were then weighted and taken in beakers. There were three replications, one of them was controlled and other two were treated by plasma for 4 min and 1 min at 800 V. Minimum 20 specimens of rust red flour beetle were released in each beaker. Beakers were covered with muslin cloth. After 15 days, these treated wheat seeds were weighed. This complete procedure was repeated three times and the percentage of weight loss was calculated with the help of formula by Padin *et al* [24].

3.3. Repellency test

This test was carried by following the method used by Khan *et al* [25] and Talukder *et al* [26] with a few modifications. Whatmann No.1 filter paper was taken and marked into two equal halves. One half was further divided into two equal halves and was marked. Another half-controlled wheat was taken, and other two treated wheat seeds were taken on other two halves. Then, 20 rust red flour beetles were released at the center of the filter paper circle; the insects were then allowed to settle down. Following this, beetles were counted at intervals of 1 h, 2 h, 3 h, 4 h and 5 h that had three replications for it [27].

3.4. Mortality test

Treated wheat seeds and controlled seeds were taken in beakers and then 20 specimens were released in each beaker having treated seeds. There were three replications of this process. After the interval of 15 days, dead specimens were counted. This procedure was repeated three times.

3.5. Surface analysis

The surfaces of untreated and plasma-treated wheat seeds were analyzed using a scanning electron microscopy (SEM) (JEOL, Japan) to study their morphological properties.

4. Results and discussion

Many methods have been used for the heat treatment of pre and post sowing of seeds such as hot air, infrared radio frequency heating, with no chemical effects and pollution free technologies. RF has low treatment time and no contamination effects as compared to other heating technologies. RF technologies with 13.56 MHz was used for a non-chemical technique for pre and post sowing of insect control in agricultural products due to rapid volumetric heating and quality maintenance.

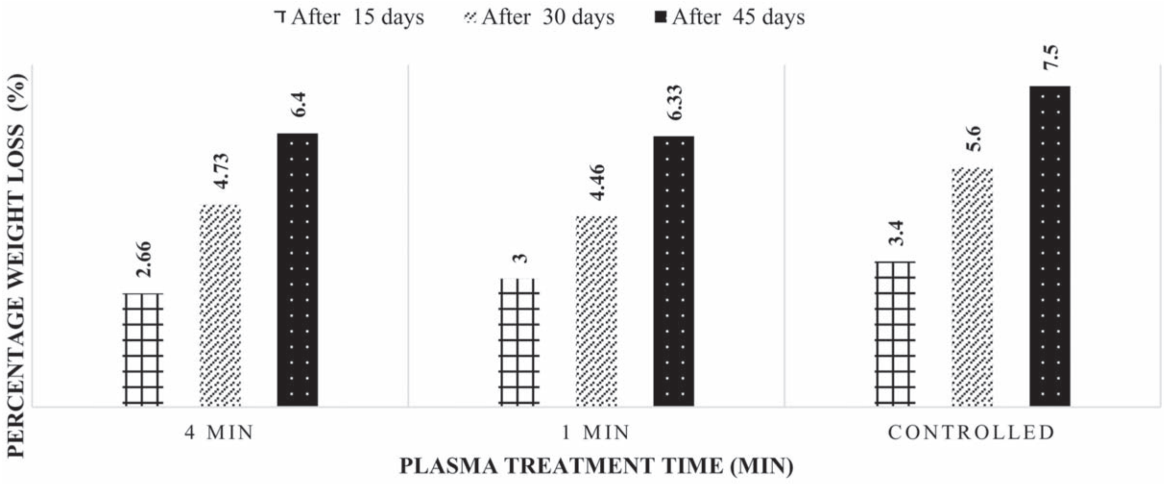

Figure 1. Variation in percentage weight loss for plasma treated seeds at different treatment time.

Table 1. Percentage weight loss and descriptive statistics of grain weight loss.

	Reduced weight			Percentage weight loss			S.D.		
Treatment time	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
4 min	0.2 g	0.24 g	0.32 g	2.66%	4.73%	6.4%	±0.016	±0.038	±0.02
1 min	0.15 g	0.23 g	0.4 g	3%	4.46%	6.33%	±0.01	±0.0058	±0.02
Controlled	0.27 g	0.28 g	0.4 g	3.4%	5.6%	7.53%	±0.01	±0.01	±0.0058

4.1. Grain weight loss

Weight losses of plasma treated, and controlled seeds were calculated as function of treatment time and applied voltage. Maximum weight loss was found in controlled wheat grains while weight loss was almost equal for both 1 min and 4 min treated seeds. After the first 15 days, weight loss was much significant while it decreased with the passage of time showing that red floor beetles have lessened the affinity with time for wheat seeds when treated with CP.

In stored grain weight loss tests, the highest of the weight loss was found in the case of controlled wheat grains, as shown in table 1. The grain weight loss in the control after 15, 30 and 45 days was 0.27, 0.28 and 0.4 g, respectively. Whereas, the grain weight loss for the seeds treated of 4 min and 1 min after 15 days was 0.2 g and 0.15 g, respectively. The lowest weight loss was in the 4 min treated wheat grains. Grain weight loss for the 4 min and 1 min treatments after 30 days was 0.24 g and 0.23 g, respectively. Grain weight loss for the 4 min and 1 min treatments after 45 days was 0.32 g and 0.4 g, respectively.

The statistical analysis was carried out on the means to find a significant difference between the mean error. In the 4 min plasma treatment, results were collected after 15, 30 and 45 days for mean reduction in the weight loss. For 15 days, the weight loss was 0.2 g with the S.D. (standard deviation) of 0.016, for 30 days the mean reduction was 0.24 g with the S.D. of 0.038 and for 45 days mean reduction was 0.32 g with S.D. of 0.02. From these results, it was observed that the seeds which were plasma at the time interval of 4 min show a significant decrease in the weight loss after passage of 45 days. It indicates that plasma was persistent and shows better results after some time. In the same way, results have also been collected for 1 min treatment after 15, 30 and 45 days. Mean reduction in weight after 14 days was 0.15 g with the S.D. of 0.01. After 30 days, mean reduction in weight was 0.23 g with S.D. of 0.0058. After 45 days, mean reduction in weight was 0.4 g with the S.D. of 0.02. This shows that maximum weight loss reduction was after 45 days. For controlled wheat grains results were also collected after 15, 30 and 45 days. After 15 days mean reduction in weight was 0.27 with S.D. of 0.01. After 30 days mean reduction in weigh was 0.28 with S.D. of 0.01. After 45 days mean reduction in weight was 0.4 with S.D. of 0.0058. This shows that weight loss reduction was decreased with the passage of time as can be seen in figure 1. Highest percentage weight loss was in the controlled after 45 days. ANOVA is a statistical analysis of variables, in which it was supposed that all values of the means were not equal. Basically, meaning all groups were compared. This value was below 0.002 and was significant.

4.2. Repellency test

In order to test the repellency test, beetles were released at the center and checked at 1 h interval. It was observed during this process that maximum individual beetles moved towards the controlled seed for every time the insects were counted. It was also observed that more insects moved towards 1 min rather than towards the 4 min treated wheat seeds. With the passage

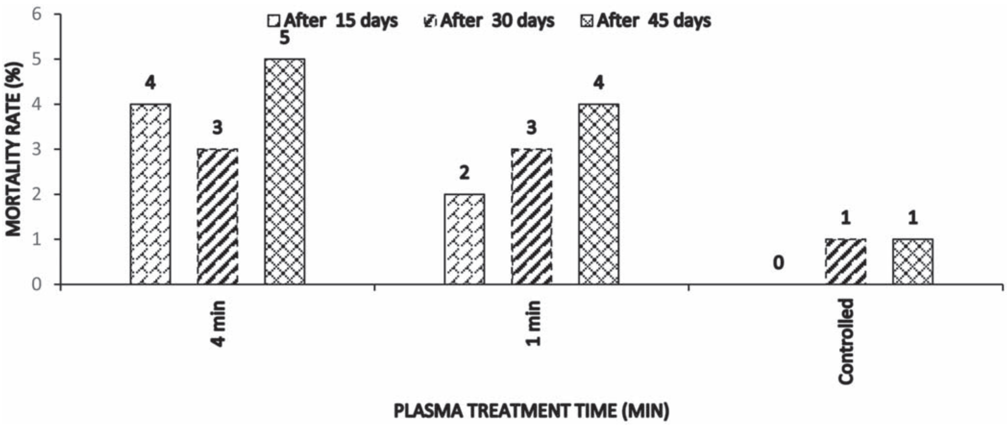

Figure 2. Mortality test of controlled seeds, for plasma treated seeds for 1 min and 4 min checked after 15, 30 and 45 days.

Table 2. Descriptive statistics of repellency test for controlled and plasma treated seed from 1–5 h (hours).

		1 h	2 h	3 h	4 h	5 h	%N	PR
Group 1	4	3	4	2	3	2	14	−72
	Controlled	11	10	10	12	12	65	10
Group 2	1	5	5	6	5	4	25	−50
	Controlled	11	11	12	13	11	58	16

of time, the number of insects moving towards the 4 min and 1 min started decreasing. At a particular stage, the same number of insects were counted in 1 min treated wheat seeds. Therefore, the 1 min treatment does not have a high insecticidal effect against red flour beetles.

In table 2, the percentage insects were shown by %N that were found in controlled and treated samples and PR shows the percentage repellency. If the value is negative, the treatment has a repellent effect and for positive values this indicates that treatment has no effect on it.

4.3. Mortality test

Mortality test shows that minimum individuals were killed in controlled wheat grains while maximum in treated wheat seeds. Between them, more individuals were killed at the 4 min treatment and the least number of individuals at 1 min treatment, as shown in table 3. This shows that plasma treatment has insecticidal effects on wheat grains, and hence can control red floor beetles in wheat.

The mortality rate for 4 min treatment after 15, 30 and 45 days was observed. The mean mortality after 15, 30 and 45 days was 4, 3 and 5 with S.D. of 0.58, 1.15 and 1, respectively. Mean mortality for 1 min treatment after 15, 30 and 45 days was 2, 3 and 4 with S.D. of 1, 0.58 and 0.58 respectively. Mean mortality for controlled after 15, 30 and 45 days was 0, 1 and 1 with S.D. of 0, 0.57 and 0.58, respectively. This shows that minimum mortality was in controlled individuals while maximum was in the 4 min plasma treatment.

Figure 2 shows the maximum mortality rate in the controlled, which was after 45 days and the minimum was in the case of 4 min which was after 15 days. This shows that plasma treatment has a similar effect for both 4 min and 1 min without significant changes from the controlled seeds.

Table 3. Descriptive statistics for mortality test.

Plasma treatment time	Reduced weight	Mean (g)	Standard deviation (S.D.)
4 min	After 15 days	4	±0.58
	After 30 days	3	±1.15
	After 45 days	5	±1
1 min	After 15 days	2	±1
	After 30 days	3	±0.58
	After 45 days	4	±0.58
Controlled	After 15 days	0	±0
	After 30 days	1	±0.57
	After 45 days	1	±0.58

4.4. Morphological study of irradiated seeds

While observing surface structure of irradiated and controlled seeds, it was found that controlled seeds have smooth and regular surface structures, as shown in figure 3. The SEM images of plasma treated seeds for 1 min were observed to be broken at different places. This breakage was clearer in 4 min plasma treatment producing irregularity on surface of seeds.

The breakdown of surface increases absorption level of seeds as more surface area is available for exchange of materials necessary for early growth of seeds e.g. water, oxygen, nitrogen and so on. The increase in absorption level enhances the water absorption of seeds. The increase in

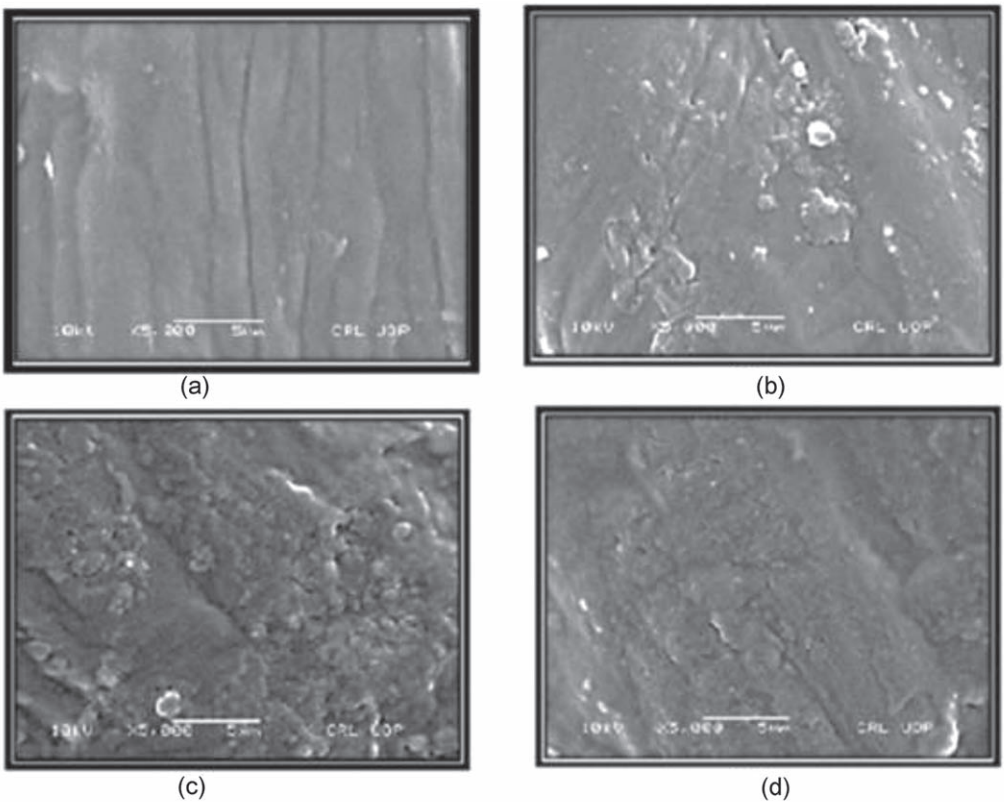

Figure 3. SEM Images of (a) controlled, (b) 1 min, (c) 4 min and (d) treated seeds.

water absorption caused early growth and enlarged root to stem ratio.

5. Conclusion

The CP treatment in agriculture is to offer novel ways for crop protection from insecticidal effects for long term storage protection. Low temperature plasma effectively had the eco environmentally agent and not any polluted solution leftovers. It was observed that weight loss of CP treated wheat seeds is very small as compared to controlled seeds which were very pronounced after 15, 30 and 45 days. Mortality test shows maximum insects were also killed in 4 min treatment and it was more persistent with the passage of time as compared to the controlled seed. The repellent effect was also very small for plasma treated seeds as compared to controlled seeds, especially for the treatment time of 4 min. So, it is concluded that CP treatment could have very effective insecticidal properties of wheat seeds against red flour beetles. This plasma-biological alliance could be used to promote the emerging technologies to sterilize and protect the agricultural and biological product.

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