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Studies on Effect of *A. niger* on Physiological Weight Loss and Biochemical Changes in Black Mould Rot Diseased Onion

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Abstract

Black mould rot of onion is an important post-harvest diseases of onion which effects on the quality deterioration of onion bulbs by utilizing its nutrient contents. Here, present research was carried out to study the losses of different nutrients from onion bulbs after the infection of *A. niger* in three different varieties. Onion bulbs inoculated with *A. niger* showed loss in weight as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation. Highest per cent physiological weight loss was recorded in Gujarat Anand white onion-3 variety (10.48 g and 16.70%) followed by Nasik yellow (11.54 g and 14.45%) and Nasik red (11.73 g and 10.59%) after 90 DAI. Total soluble sugar content in onion bulbs inoculated with *A. niger* progressively decreased as the incubation period was increased over control in all three varieties. Least TSS (10.90, 6.39 and 6.09%) and reducing sugar content (1.81, 1.13 and 1.09%) was recorded in onion bulbs after 90 DAI over control (18.21, 17.25 and 14.11%); (6.91, 5.48 and 5.23%) in Nasik red, yellow and Gujarat Anand white onion-3 varieties, respectively. There was appreciable decrease in phenol content in onion bulbs inoculated with *A. niger* up to 60 DAI due to utilization by fungi and then there was increase in total phenol content in onion bulbs up to 90 DAI when inoculated with *A. niger*. Total phenol content of healthy Nasik red, yellow and Gujarat Anand white onion-3 varieties were 51.23, 46.07 and 38.78 per cent, which showed maximum reduction after 60 DAI (30.69, 27.36 and 20.24%) and found increased after 90 DAI (33.72, 29.60 and 26.22%). Significantly lowest amount of ascorbic acid content was recorded at 90 DAI (0.03, 0.014 and 0.014 mg/100g) over control (0.14, 0.14 and 0.13 mg/100g), least pyruvic acid content was found at 90 DAI (257.82, 217.11 and 158.65 mg/100 g) over control (755.48, 589.77 and 512.78 mg/100 g), least acidity content was found at 90 DAI (2.05, 2.81 and 1.28%) over control (6.40, 5.76 and 3.84%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties, respectively.

Keywords

Onion; *Aspergillus niger*; Total sugar; Reducing sugar; Phenol; Ascorbic acid; Pyruvic acid; Acidity content

Highlight

Onion bulbs inoculated with *A. niger* showed loss in weight, loss in total sugar content, reducing sugar content, ascorbic acid content, pyruvic acid content and acidity content as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation. While, phenol content in onion bulbs inoculated with *A. niger* showed decrease up to 60 days after inoculation and after that it showed increased content up to 90 days.

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Introduction

Onion (*Allium cepa* L.) is an important vegetable crop widely cultivated and used in the world. It is pungent when chopped and contains sulfenic acid which irritates the eyes with tears [1]. Onion contain phenolics and flavonoides which has potential anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties. Most onion cultivars contains about 89% water, 4% sugar, 1% protein, 2% fiber and 0.1% fat, vitamin C, vitamin B_6, folic acid and numerous other nutrients in small quantity. They are low in fats and sodium. They can contribute their flavor to savory dishes without increasing caloric content [1]. After harvest of the bulbs when they are preserved for longer period, many fungal and bacterial pathogens cause deterioration of bulbs. Under storage conditions these fungal rotting cause heavy bulb losses. Black mould rot is the most destructive disease of onion in storage as well as under field conditions [2]. Raju and Naik [3] reported 16.52 and 14.76 per cent disease incidence of black mould rot of onion in Gulbarga and Bellary vegetable market, respectively. Nanda et al., [4] estimated 7.51 per cent overall post-harvest losses in *kharif* onion. Storage losses of *Rabi* harvested onions ranged from 10-15 per cent due to microbial decay or rotting due to fungal diseases [5]. The present research work was undertaken to study the effect of *Aspergillus niger* infection on physiological weight loss and biochemical changes in onion bulbs.

Materials and Methods

Collection and identification of the pathogen

Fresh naturally infected diseased onion bulbs showing typical characteristic symptoms of black mould rot were collected from the Sardar Patel vegetable market, Anand and brought to the laboratory for isolation of the pathogen. Pathogenicity was proved by following Koch's postulates. The pure culture obtained was sent for identification to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi and was identified as *A. niger* (ID. No. 9610.14).

Physiological weight loss

Mature, healthy uniform size onion bulbs of three varieties *i.e.*, Nasik Red, Nasik Yellow and Gujarat Anand White Onion-3; were washed with tap water and then surface sterilized with 1 per cent NaOCl and finally washed thoroughly with distilled sterile water and separately inoculated with *A. niger* (10^6 spores/ml) by random pin-pricking method. The inoculated bulbs were incubated at ambient temperature. Physiological weight loss of infected bulbs was assessed after 30th, 60th and 90th days and losses in weight were calculated by the following formula:

$$= \frac{W_1 - W_2}{W_1} * 100$$

Where,

W_1 = The weight of bulb recorded at the time of inoculation

W_2= The weight of bulb recorded after 30th, 60th and 90th days after inoculation

Total soluble sugar content: Total soluble sugar content of inoculated and un-inoculated mature onion bulbs was determined by phenol sulphuric acid method as described by Dubois [6].

Reducing sugar content: Reducing sugar content of inoculated and un-inoculated mature onion bulbs was determined by the method of Nelson's modification of Somogyi's method [7,8].

Total phenol content: Total phenol content of inoculated and un-inoculated mature onion bulbs both was estimated by Folin ciocalteau method as described by Bhatnagar et al. [9].

Ascorbic acid content: The titramatric method described by Ranganna [10] was adopted to estimate the ascorbic acid content.

Pyruvic acid content: The DNPH (dinitrophenylhydrazine) method described by Anthon and Barrett [11] was employed to estimate the pyruvic acid content.

Acidity content: The method described by Ranganna [10] was employed to estimate the acidity.

Results and Discussion

Physiological weight loss

The results presented in Table 1 revealed that onion bulbs inoculated with *Aspergillus niger* showed loss in weight as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation. Highest per cent physiological weight loss was recorded in Gujarat Anand white onion-3 variety (16.70%) followed by Nasik yellow (14.45%) and red (10.59%), after 90 days of inoculation (DAI). It was observed that physiological weight of onion bulbs decreased quickly when inoculated with *A. niger* as compared to control bulbs. The results of present investigation corroborate with the results obtained by Kapadiya et al., [12]. They reported maximum weight loss (5.44%) in black mould rot infected onion bulbs as compare to healthy bulbs (2.66%) through fresh neck cutting injury method. Damaram [13] reported that when tomato fruits are inoculated with *F. pallidoroseum* showed drastic loss in physiological weight compared to uninoculated healthy fruits. Highest physiological weight loss was recorded after 8th day of inoculation.

Total soluble sugar content

The results of total sugar content in onion bulbs inoculated with *Aspergillus niger* and control (without pathogen) at different incubation periods are given in Table 2. The results revealed that TSS in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control in all three (Nasik red, yellow and Gujarat Anand white onion-3 varieties). Least TSS content was found after 90 DAI (10.90, 6.39 and 6.09%) followed by 60 DAI (12.69, 10.76 and 10.33%) and 30 DAI (16.14, 15.51 and 13.20%) over control (18.21, 17.25 and 14.11%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties. The results of present investigation are in consonance with the results obtained by Ghangaonkar [14]. He reported that *A. niger* significantly utilized total sugar contents from red and white onion varieties. Further Singh and Sinha [15] found that *A. flavus* and *A. parsiticus* resulted in depletion of total sugars from guava fruit. Rathod and Chavan [16] reported maximum reduction in total sugar content of papaya fruits due to *A. niger*. Bilgrami et al., [17] revealed that there was sharp decline in the level of total sugars of dry fruit during infection by *A. flavus*.

Reducing sugar content

The results of reducing sugar content in onion bulbs inoculated with *A. niger* and control (without pathogen) after different periods of incubation are given in Table 2. The results revealed that reducing sugar content in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control in all the three varieties. Least reducing sugar content was recorded after 90 DAI (1.81, 1.13 and 1.09%) followed by 60 (2.37. 2.31 and 2.71%) and 30 DAI (4.31, 4.07 and 4.10%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties over control (6.91, 5.48 and 5.23%), respectively. It was observed that reducing sugar content of onion bulb decreased when inoculated with *A. niger* as compared to control bulbs. Results of present investigation corroborate with the results obtained by Singh and Sinha [15]. They reported that *A. flavus* and *A.*

Table 1: Physiological weight loss in different onion varieties inoculated with *A. niger*.

Sr. No.	Treatments	Initial bulb wt. (g) at the period			Bulb wt. (g) after inoculation at different periods			Physiological loss in weight			Per cent physiological loss in weight		
		30 days	60 days	90 days	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
1.	Nasik red	106.27	109.2	110.71	104.29	103.48	98.98	1.98	5.72	11.73	1.86	5.24	10.59
2.	Nasik yellow	64.7	63.48	79.85	62.74	58.10	68.31	1.96	5.38	11.54	3.03	8.47	14.45
3.	Gujarat Anand White onion-3	55.26	59.72	62.74	52.77	52.87	52.26	2.49	6.85	10.48	4.51	11.47	16.70

***DAI= Days after inoculation**

Table 2: Effect of *A. niger* infection on total sugar, reducing sugar and phenol contents of different onion varieties

Sr. No.	Treatments	Total sugar (%)			Reducing sugar (%)			Total phenol (mg/100g)		
		Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3
1.	Control	18.21	17.25	14.11	6.91	5.48	5.23	51.23	46.07	38.78
2.	30 DAI	16.14	15.51	13.20	4.31	4.07	4.10	43.41	37.53	30.95
3.	60 DAI	12.69	10.76	10.33	2.37	2.31	2.71	30.69	27.36	20.24
4.	90 DAI	10.90	06.39	06.09	1.81	1.13	1.09	33.72	29.60	26.22
SEm±		0.031	0.088	0.045	0.011	0.013	0.015	0.312	0.602	0.387
C.D. (5 %)		0.092	0.264	0.135	0.033	0.038	0.046	0.935	1.804	1.161
C.V. %		0.472	1.579	0.922	0.632	0.868	1.042	1.753	3.839	2.980

parsiticus resulted in depletion of reducing and non-reducing sugars from guava fruit. Rajmane and Korekar [18] reported maximum decrease in reducing sugar in local varieties of mango (Nanded, Aurangabad) when infected by *A. niger*. Rathod and Chavan [16] reported maximum reduction in reducing and non-reducing sugar content in papaya fruits due to infection by *A. niger*. Bilgrami et al., [17] revealed that there was sharp decline in the level of reducing and non-reducing sugars of dry fruit during *A. flavus* infection.

Total phenol content

The results revealed that there was appreciable decrease in phenol content up to 60 DAI due to utilization by fungi and then there was increase in total phenol content in onion bulbs up to 90 DAI when inoculated with *A. niger*. Total phenol content of healthy Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties was 51.23, 46.07 and 38.78 per cent, which showed maximum decrease after 60 DAI (30.69, 27.36 and 20.24%) followed by 30 DAI (43.41, 37.53 and 30.95%) and found increased after 90 DAI (33.72, 29.60 and 26.22%). Phenol accumulated in the bulbs infected by pathogen which might be inactivate the enzymes of pathogens by forming poly phenol oxydase which in turn prevented further advancement of pathogen by limiting its source of nutrients [18]. The significance of phenols in onion bulb is to trigger the resistance. Results similar to the present investigation were obtained by Ghangaonkar [14]. He reported that *A. niger* significantly utilized phenol content from red and white onion varieties. Especially phenols which are responsible for inhibiting the growth of fungi were utilized by the pathogen. The white onion variety seems to be more susceptible to rot than red. Clark and Lorbeer [19] reported that catachol (phenol) which is responsible for inhibiting the growth of fungi was absent in white onions, while it is very common in red and yellow varieties. Wagh and Bhale [20] found that phenol content was decreased significantly due to five isolates of *A. niger* in sapota.

Ascorbic acid content

The results presented in Table 3 revealed that there was appreciable decrease in ascorbic acid content as per incubation period increased in onion bulbs when inoculated with *A. niger*. Significantly lowest amount of ascorbic acid content was recorded at 90 DAI (0.03, 0.014 and 0.014 mg/100g) followed by 60 DAI (0.07, 0.07 and 0.05 mg/100g) over control (0.14, 0.14 and 0.13 mg/100g) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties. Thus, it was observed that ascorbic acid content was progressively decreased as the inoculation period is increased in inoculated onion bulbs as compared to control. The probable reason for drastic reduction in ascorbic acid content of onion bulb infected with *A. niger* could be due to changes in biochemical content of bulbs which revealed that the fungus (*A. niger*) might have utilized it as a substrate. Similar trend of the results was reported by Singh et al. [21]. They reported that ascorbic acid content of papaya fruits inoculated with *A. flavus* and *F. moniliforme* showed 71.10 and 62.10 per cent decrease in ascorbic acid content, respectively as compared to control (76.00%). Ghosh et al. [22] noted post-infectional change in ascorbic acid content of mango and papaya fruits when inoculated with *A. niger*. As the incubation progressed, there was a decrease in ascorbic acid content in both healthy as well as infected fruits. After four days of incubation the quantity of ascorbic acid content was reduced to nearly half the original amount.

Ogaraku et al. [23] carried out studies on storage decay of tomato fruits and vitamin C content in fruits inoculated with *A. niger, A. flavus, Alternaria alternata, A. solani* and *Fusarium oxysporium*. The results revealed that the infected fruit contained 2.20 mg/100 g vitamin C, while the healthy tomato fruits contained 2.51 mg/100 g vitamin C.

Pyruvic acid content

The results of pyruvic acid content presented in Table 3 revealed that pyruvic acid content in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control bulbs in all three varieties. Least pyruvic acid content was found after 90 DAI (257.82, 217.11 and 158.65 mg/100 g) followed by 60 (460.60, 349.43 and 216.43 mg/100 g) and 30 DAI (604.38, 461.37 and 361.95 mg/100 g) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 bulbs, respectively. It was observed that pyruvic acid content of onion bulb decreased when inoculated with *A. niger* as compared to control bulbs (755.48, 589.77 and 512.78 mg/100 g).

Acidity content

The results of acidity content presented in Table 3 revealed that acidity content in inoculated onion bulbs with *A. niger* progressively decreased as the incubation period was increased over control in all three varieties. Least acidity content was found after 90 DAI (2.05, 2.81 and 1.28%) followed by 60 (3.07, 4.09 and 2.56%) and 30 DAI (5.12, 4.61 and 3.33%) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties. It was observed that acidity content of onion bulb decreased when inoculated with *A. niger* as compared to control (6.40, 5.76 and 3.84%). Results of present investigation corroborate with the results obtained by Sharma et al., [24]. They reported the post-infectional changes pertaining to physical, biochemical and nutritional aspects caused due to major post-harvest microbial rot pathogens (*Alternaria alternata, Botryodiplodia theobromae, G. candidum, Penicillium digitatum* and *P. italicum*) in kinnow fruits. The results revealed significant decrease in fruit acidity and ascorbic

Table 3: Effect of *A. niger* infection on ascorbic, pyruvic acid and acidity contents of different onion varieties.

Sr. No.	Treatments	Ascorbic acid (mg/100g)			Pyruvic acid (mg/100g)			Acidity (%)		
		Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3	Nasik Red	Nasik Yellow	Gujarat Anand White onion-3
1.	Control	0.14	0.14	0.13	755.48	589.77	512.78	6.40	5.76	3.84
2.	30 DAI	0.11	0.09	0.08	604.38	461.37	361.95	5.12	4.61	3.33
3.	60 DAI	0.07	0.07	0.05	460.60	349.43	216.33	3.07	4.09	2.56
4.	90 DAI	0.03	0.014	0.014	257.82	217.11	158.65	2.05	2.81	1.28
SEm±		0.003	0.002	0.002	3.242	2.507	3.767	0.222	0.239	0.157
C.D. (5 %)		0.009	0.006	0.006	9.721	7.518	11.294	0.665	0.718	0.470
C.V. %		7.744	5.590	6.681	1.395	1.386	2.696	11.917	12.395	12.738

acid content as compare to uninoculated healthy fruits. Damaram [7] observed that acidity content of inoculated tomato fruits with *F. pallidoroseum* progressively decreased (0.78, 0.66, 0.55 and 0.48%) as the incubation period is increased (2nd, 4th, 6th and 8th day), while in control fruit acidity was 0.87 percent.

Conclusion

Onion bulbs inoculated with *A. niger* showed loss in weight as compared to uninoculated bulbs at 30, 60 and 90 days after inoculation in all three varieties. Total soluble sugar and reducing sugar content in onion bulbs inoculated with *A. niger* progressively decreased as the incubation period was increased over control in all three varieties. There was appreciable decrease in phenol content in onion bulbs inoculated with *A. niger* up to 60 DAI due to utilization by fungi and then there was increase in total phenol content in onion bulbs up to 90 DAI when inoculated with *A. niger*. This may be due to resistance mechanism of phenol. Similarly ascorbic acid, pyruvic acid and acidity content of onion bulbs infected with *A. niger* were found decreasing as the period of storage increased as compared to control in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties, respectively.

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