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Physiological weight loss and biochemical changes in garlic bulbs inoculated with *Aspergillus niger* van Teighem causing black mould rot of garlic

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Abstract
Red and white garlic bulbs were analysed to evaluate their physiological weight loss and biochemical changes in both the garlic varieties. Biochemical parameters were: total pyruvic acid content, total phenol content and total sulphur content. We found that garlic bulbs inoculated with *A. niger* showed loss in weight at 15, 30, 45 and 60 days after inoculation. While, pyruvic acid content and total sulphur content in inoculated garlic bulbs with *A. niger* progressively decreased over control bulbs in both the garlic varieties as the incubation period was increased. Phenol content increases in inoculated bulbs after 60 days of inoculation in GAG-6 and GG-4 garlic varieties.

Keywords: Garlic, physiological weight loss, pyruvic acid, total sulphur content, total phenol content, *Aspergillus niger*

Introduction
Garlic (*Allium sativum* L.) is one of the most important vegetables and mostly used as a spices in the form of bulbs fresh from field or after storage. The centre of origin of garlic was considered as Central Asia, than it spread to Asia, Europe and in America (Abedi *et al*., 2013) [1]. Today Garlic is cultivated in India as the second major bulbous crops next to onion, covering an area of 227.39 thousand hectares with a total production of 1181.37 thousand MT having productivity of 5195 kg per hectare (Anon., 2014a) [2]. Garlic is also cultivated in Gujarat over 39.20 thousand hectares with total production is about 277.46 thousand MT having productivity of 7.08 tons per hectare (Anon., 2015a) [3].
Garlic is a valuable condiment for food and consumed by most of the people. It is used all over the world for flavouring and seasoning various vegetables and meat dishes. In India and other Asian and middle-east countries, it is used in several food preparations, like chutneys, pickles, curry powders, curried vegetables, meat preparations and tomato ketchup. It is rich in protein, minerals and vitamins like vitamin B, vitamin C and fat as well (Gopalan, *et al*., 1984) [12].
Colonization of *Aspergillus niger* on garlic bulbs always resulted in decrease of bulb content, loss in weight and biochemical changes in bulbs. This phenomenon is referred as bulb deterioration. The losses due to bio-deterioration can be categorized in the form of loss in bulb weight, increase in bulb rots, biochemical changes and release of mycotoxins due to pathogen. The fungus *A. niger* causing black mold rot in garlic is known to cause drastic biochemical changes in the garlic bulb therefore to understand the changes due to infection of *A. niger* the experiments were carried out *in vitro*. For present studies Gujarat Garlic-4 (white garlic) and Gujarat Anand Garlic-6 (red garlic) varieties of garlic bulbs were analyzed comparatively.

Materials and Methods
Physiological weight loss
Healthy, mature and uniform sized garlic bulbs were surface sterilized with NaOCl (1%) and finally washed with distilled sterile water and separately inoculated with *A. niger* by pin-prick method. The inoculated bulbs were incubated at ambient temperature. Physiological losses in weight of infected bulbs were assessed on 15th, 30th, 45th and 60th day and losses in weight were calculated by the following standard formula:

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

Where,
W_1= the weight of bulb recorded at the time of inoculation
W_2= the weight of bulb recorded after 15th, 30th, 45th and 60th day after inoculation

Pyruvic acid content

Pyruvic acid content from the inoculated and uninoculated mature garlic bulb was estimated by DNPH (2, 4-dinitrophenylhydrazine) method as described by Abedi *et al*., (2013) [1].

One gram of garlic pulp was crushed with 10 ml phosphate buffer which was taken into centrifuge tubes for centrifugation at 10,000 rpm for 10 min. After centrifugation, 0.2 ml aliquot was taken from supernatant in the test tubes. To this one ml each of distilled water and 0.5 ml of 2, 4-dinitrophenylhydrazine (DNPH) was added. After half an hour, 5 ml of sodium hydroxide (NaOH) was added. After 10 min, intensity of colour was recorded at 510 nm in spectrophotometer. Blank was prepared by taking one ml distilled water and the rest of the procedure was followed as described earlier for sample estimation as above.

Total phenol content

Total phenol content from the garlic bulb both inoculated and un-inoculated was estimated by Folin ciocalteau method as described by Bhatnagar *et al.* (2005) [4]. 50 mg of pulp was crushed with 25 ml of 0.3 N HCl which was kept for shaking for about one hour. After shaking, crude extract was centrifuged at 8000 rpm for about 10 min. One ml supernatant obtained was evaporated to dryness on water bath. To the residue hot water was added and final volume was adjusted to 250 ml with distilled water in volumetric flask. One ml of the above aliquot was taken in the test tube. To this one ml each of Folin – Ciocalteau reagents diluted 1: 2 and 1 ml of sodium carbonate (35%) was added. After one hour 2 ml distilled water was added to adjust the final volume (5 ml). Intensity of the colour was recorded at 620 nm in spectrophotometer. Blank was prepared by taking one ml distilled water.

Total sulphur content

Total sulphur content of inoculated and un-inoculated garlic bulbs both was estimated by tubiditory method as described by Blancher *et al.*, (1965) [5]. 50 mg of garlic pulp was crushed with 3 ml of conc. HNO_3 which was pre-digested overnight over a hot plate at 100°C. 5 ml HNO_3: $HClO_4$ mixture was added and digestion at high temperature was continued till a white oilish residue was left out. 5 ml of above acid extract was taken in 50 ml volumetric flask. To this 20 ml of Morgan's reagent and 2 ml of gum acacia was added to each of the flask and was diluted to about 45 ml with distilled water. Flask was shaken for one minute and 1 g barium chloride powder was added to each of the flask. Distilled water was added to make the volume of each flask to 50 ml and shaken intermittently for 3 minutes. After 20 min turbidity was measured on spectrophotometer using 430 nm.

Results and Discussions

Physiological weight loss

The results revealed that garlic bulbs inoculated with *A. niger* showed loss in weight as compared to uninoculated bulbs at 15, 30, 45 and 90 days after inoculation. Highest per cent physiological weight loss was recorded in Gujarat Garlic-4 variety (4.87 g & 29.53%) followed by Gujarat Anand Garlic-6 variety (4.38 g & 28.77%), after 60 days of inoculation (DAI). It was observed that physiological weight of garlic bulbs progressively decreased as the incubation period is increased over control (Table-1).

The results of present investigation are in agreement with the results obtained by Maini and Chakravarti (2000) [14]. They reported 12-25 per cent losses in physiological weight due to various diseases such as soft rot, blue mold and black mold rot. Total losses during storage of 5 – 6 months were as high as 30 – 40 per cent. Chundawat *et al*. (1976) [8] reported that guava fruits when infected with *A. niger*, *Botryodiplodia theobromae*, *Pestalotia psidii*, *Cladosporium tenuissimum*, *Glomerella cingulata*, *A. flavus*, *A. carbonarious* and *F. solani* had more enzymatic weight loss as compared to uninoculated fruits. Kapadiya *et al*. (2013) [13] reported maximum weight loss (5.44%) in black mold rot infected onion bulbs as compare to healthy bulbs (2.66%) through fresh neck cutting injury method. Damaram (2013) [10] reported that when tomato fruits were inoculated with *F. pallidoroseum* showed drastic loss in physiological weight compared to uninoculated healthy fruits. Highest physiological weight loss was recorded after 8th day (35.15 g & 50.21%) of inoculation followed by 6th (28.73 g & 41.04%) and 4th (17.15 g & 24.50%) day after inoculation. It was observed that physiological weight of tomato fruits decreased when inoculated with *F. pallidoroseum* as compared to control fruits (70.00 g).

Pyruvic acid content

The results of pyruvic acid, total phenol and total sulphur content in garlic bulbs inoculated with *A. niger* and control (without pathogen) at different incubation periods are given in Table-2. The results revealed that pyruvic acid content in inoculated garlic bulbs with *A. niger* progressively decreased as the incubation period was increased over control in both the garlic varieties. Least pyruvic acid content was found after 60 DAI (112.87 & 107.65 mg/100 g) followed by 45 DAI (151.36 & 142.22 mg/100 g), 30 DAI (209.42 & 194.42 mg/100 g) and 15 DAI (303.37 & 230.95 mg/100 g) in GAG-6 and GG-4 varieties. It was observed that pyruvic acid content of garlic bulb decreased when inoculated with *A. niger* as compared to control (369.26 & 294.23 mg/100 g).

Results similar to the present investigation were obtained by Prajapati (2016) [15]. He reported pyruvic acid content in onion bulbs inoculated with *A. niger* progressively decreased as the incubation period was increased over control in all the three onion varieties. Least pyruvic acid content was found at 90DAI (257.82, 217.11 & 158.65 mg/100 g) followed by 60 DAI (460.60, 349.43 & 216.43 mg/100 g) and 30 DAI (604.38, 461.37 & 361.95 mg/100 g) over control (755.48, 589.77 & 512.78 mg/100 g) in Nasik red, Nasik yellow and Gujarat Anand white onion-3 varieties.

Total phenol content

The results of total phenol content in garlic bulbs inoculated with *A. niger* were compared with that of control (uninoculated) at different periods in both the garlic varieties. The results revealed that there was an appreciable increase in total phenol content in garlic bulbs up to 60 DAI when inoculated with *A. niger*. Total phenol content of healthy red and white garlic bulbs was 38.90 and 37.60 per cent, respectively, which showed maximum increase after 15 DAI (39.62 & 38.17 mg/100 g) followed by 30 DAI (41.27 & 40.70 mg/ 100 g), 45 DAI (44.94 & 44.61 mg/ 100 g) and 60 DAI (55.79 & 56.76 mg/ 100 g). Phenol accumulated in the bulbs infected by pathogen which might be inactivated the enzymes of pathogens by forming poly phenol oxydase which in turn prevented further advancement of pathogen by limiting its source of nutrients. The significance of phenols in garlic bulb is to trigger the resistance.

Results similar to the present investigation were obtained by Ghangaonkar (2013) [11]. 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 (1973) [9] reported that Catechol (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. Srivastava and Kumar (2013) [17] studied the phenol content in onion bulbs inoculated with *Botrytis* sp. The results revealed that phenol level was high in infected onion as compared to fresh onion.

Total sulphur content

The results of total sulphur content in garlic bulbs inoculated with *A. niger* and control bulbs (without pathogen) at different incubation periods are given in Table-2. The results revealed that sulphur content in inoculated garlic bulbs progressively decreased over control as the incubation period increased in both the garlic varieties. Lowest sulphur content was found in 60 DAI (0.51 & 0.44%) followed by 45 DAI (0.66 & 0.64%), 30 DAI (0.77 & 0.72%) and 15 DAI (0.81 & 0.75%) in GAG-6 and GG-4 varieties, respectively. It was observed that sulphur content of garlic bulb decreased when inoculated with *A. niger* as compared to control bulbs (0.85 & 0.79%).

Results similar to the present findings were obtained by Bloem *et al*. (2015) [7]. They studied impact of sulphur nutrition and fungal infection on *Sclerotinia sclerotiorum* and reported that the infection of *S. sclerotiorum* caused significant decrease in the sulphur content of rapeseed crop. Raj and Srivastava (1977) [16] showed that the total sulphur content of infected tissue of *mustard crop* was inversely correlated with the pathogenicity of different isolates of *Macrophomina phaseolina* and suggested that the pathogens are able to metabolize sulphur from the host plant. Bloem *et al*. (2007) [6] reported that the reduction of sulphur content in grapes due to infection of *Uncinula necator* which increase the release of H_2S after fungal infection.

Table 1: Physiological weight loss of garlic bulbs inoculated with *A. niger*

Sr. No.	Variety	Initial bulb wt. (g) at the period				Bulb wt. (g) after inoculation at				Physiological weight loss (g)				Per cent physiological weight loss			
		15 Days	30 Days	45 Days	60 Days	15 DAI*	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI
1	Gujarat Garlic – 4 (White Garlic)	16.00	16.35	17.24	16.49	15.73	14.91	13.85	11.62	0.27	1.44	3.39	4.87	1.68	8.80	19.66	29.53
2	Gujarat Anand Garlic – 6 (Red Garlic)	15.42	15.86	14.93	15.22	14.98	14.02	12.07	10.84	0.44	1.84	2.86	4.38	2.85	11.60	19.15	28.77

* DAI = Days after inoculation

Table 2: Effect of *A. niger* infection on pyruvic acid, phenol and sulphur contents of garlic bulbs

Sr. No.	Periods	Pyruvic acid (mg/100g)			Total phenol (mg/100g)			Total Sulphur (%)		
		*GAG-6		**GG-4	GAG-6		GG-4	GAG-6		GG-4
1	15th day	303.37		230.95	39.62		38.17	0.81		0.75
2	30th day	209.42		194.42	41.27		40.70	0.77		0.72
3	45th day	151.36		142.22	44.94		44.61	0.66		0.64
4	60th day	112.87		107.65	55.79		56.76	0.51		0.44
5	Control	369.26		294.23	38.90		37.60	0.85		0.79
		V*	P**	V X P	V	P	V X P	V	P	V X P
	SEm±	0.79	1.24	1.76	0.08	0.12	0.17	0.00	0.01	0.01
	C.D. at (5%)	2.24	3.55	5.02	0.22	0.34	0.48	0.01	0.01	0.02
	C.V. %	1.86			0.87			1.84		

*V= Variety, **P=Period

*GAG-6= Red Garlic, **GG-4= White Garlic

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References

1. Abedi M, Biat F, Nosrati AE. 2013.
2. Anonymous. Agricultural statistics at a glance 2014, Dept. of Agril & Cooperation (Horticulture wing), 2014a.
3. Anonymous. State of Indian Agriculture, Government of India, Ministry of Agriculture and Cooperation, New Delhi, 2015a.
4. Bhatnagar R, Shukla YM, Talati JG. Biochemical methods for agricultural sciences, Anand Agricultural University., Department of Biochemistry, 2005, 184p.
5. Blanchar RW, Rehm G, Coldwell AC. Soil Sci Soc America Proceed. 1965; 29(1):71-72.
6. Bloem E, Haneklaus S, Kesselmeier J, Schnug E. Plant Biol (Stuttg). 2007; 9(5):596-607.
7. Bloem E, Haneklaus S, Schnug E. Front Plant Sci. 2015; 5:779.
8. Chundawat BS, Singh JP, Kamsa R, Gupata OP. Haryana J Horti. Sci. 1976; 5:130-136.
9. Clark CA, Lorbeer JW. Phytopath. 1973; 65(3):338-341.
10. Damaram. M. Sc. (Agri.) Thesis submitted to Anand Agricultural University, Anand, Gujarat, 2013, 64-65p.
11. Ghangaonkar NM. An International Peer-reviewed Journal. 2013; 2(1):7-8.
12. Gopalan C, Rana BV, Sastri SC. ICMR, Hyderabad, 1984, 48-49p.
13. Kapadiya HJ, Pathak DM, Patel DR. Int. J Plant Prot. 2013; 6(2):422-424.
14. Maini SB, Chakravatri AK. Challenges and strategies, (Pp. 24-32) NHRDF, Nasik, 2000.
15. Prajapati BK. M. Sc. (Agri.) Thesis submitted to Anand Agricultural University, Anand, Gujarat, 2016.

16. Raj JN, Srivastava SK. Ind. Phytopathol. 1977; 30:486-488.
17. Srivastava A, Kumar S. IOSR J Agri. Vet. Sci. 2013; 5(4):18-21.