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Research Article

EFFECT OF APHID INFESTATION ON WHEAT QUALITY

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Abstract: Wheat (*Triticum aestivum* L.) is the one of the most important cereal crops of world. The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. However, the nutritional value of wheat grains is often reduced due to various biotic stresses, mainly through pest attack. Aphids are the single most important insect pest causing significant reduction in yield and quality of wheat grains. This study aims to provide experimental evidence for the various nutritional losses caused by aphid feeding in wheat grains. Different bread wheat varieties (PBW 621 and HD 2967 (Timely sown irrigated), PBW 590 and PBW 658 (Late sown irrigated), and PBW 644 and PBW 660 (Timely sown rainfed)) were grown in two plots each, one under the aphid infested and other under uninfested condition. Matured grains thus obtained after harvesting were collected, cleaned and various quality parameters such as grain appearance score, test weight, grain hardness grain plumpness, SDS sedimentation, starch parting characteristics was analyzed. All the physical as well as chemical parameters were adversely affected by the aphid infestation.

Keywords: Aphid infestation, Host resistance, Nutrient deterioration, Quality parameters, Wheat grains

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Introduction

Wheat is the principal winter cereal crop of India and it was grown over 31.34 million hectares with an annual production of 95.91 million tonnes of food grains [1]. Insect pest attack is one of the major issues in wheat production and an annual loss of Rs 413.68 billion has been reported due to insect pests in wheat in India [2]. A number of insect pests attack wheat crop throughout the season, out of which aphids are considered as the major pest in North-western plains of India. A complex of five species *i.e. Sitobion miscanthi, S. avenae, Rhopalosiphum padi, R. maidis* and *Schizaphis graminum* infest wheat crop in this part of the country, out of which *S. micanthi* and *S. avenae* caused significant damage to developing grains. The aphid damage is seen during grain filling stage when both nymphs and adults take a heavy toll by sucking cell sap from leaves and maturing grains. The infested leaves turn pale, wilt and wear a stunted appearance and cause 3-21 per cent grain yield loss [3]. Aphids also caused significant changes in the quality of wheat grains. Aphids particularly damage wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Symptoms of aphid feeding include leaf chlorosis, plant stunting, leaf rolling, and plant desiccation, and these ultimately lead to yield reduction [4]. Bread is a staple food many countries and it is rich source of protein and provides a daily requirement of energy. Wheat is rich in manganese, phosphorus, magnesium, vitamins and selenium. The effect of aphid feeding on grain quality has been studied in wheat. When present on the crop before flowering, the aphids damage the crop by reducing the numbers of grains in the ear; when present from flowering to the end of the grain-filling period, the aphids reduce the size of the grain [5]. Grain aphids infest both the ear and the upper leaves. Aphids are reported to reduce both the protein content and dough mixing time of flour of susceptible wheat varieties [6]. A significant reduction in the percentage of epicuticular wax, dry weight, sugar, amino acids levels were also found with aphid feeding [7]. Furthermore, aphid herbivory elicits an effect on the uptake of some nutrient elements such as P, K, Ca, Mg and Fe. Bread-making quality of wheat flour is determined primarily by the protein content, the gluten proteins (gliadins, glutenins) being the prime factors [8].

The gliadin/glutenin ratio was significantly lowered in flour made from aphid-infected wheat seeds, thus it could be supposed that aphid feeding resulted in decreased bread making quality of wheat flour [6]. In South Africa, wheat grain yield loss on individual susceptible plants has been reported to be as high as 90% [9] and in Kenya, up to 90 % yield loss in wheat grains was due to aphid infestation [10]. Keeping in mind the importance of maintaining wheat quality and paucity of knowledge, field and laboratory investigations were carried out to observe the changes in quality of wheat grains in relation to aphid infestation in wheat.

Materials and Methods

Experimental design and sampling methods: This field experiments were conducted under irrigated conditions at Plant Breeding Research Farm, Punjab Agricultural University, Ludhiana (30°55' N 75°54' E and 250 m above sea level) during 2014-15. Six bread wheat varieties PBW 621 and HD 2967 (timely sown irrigated), PBW 590 and PBW 658 (late sown irrigated), PBW 644 and PBW 660 (timely sown rainfed) were sown in factorial randomized complete block design (RCBD) under insecticide protected and unprotected conditions. Insecticide protection and genotypes were the two factors and each treatment were replicated thrice in experimentation. Imidacloprid (confidor 17.8 SL) was sprayed to control aphids in insecticide protected conditions and kept as control. Whereas other plot was not sprayed to observe the biochemical changes caused by the attack of aphid complex under the natural conditions. The crop was raised by following recommended standard package of practices except for the usage of pesticides [11]. Mature grain samples from infested and uninfested plants of all the test genotypes were collected after harvest and evaluated for grain quality parameters. The samples drawn from the produce of infested and uninfested plants of each genotype were cleaned and stored in airtight containers till further use. The whole meal (atta) for use in the chemical analysis, pasting characteristics and gluten content, was produced on a laboratory grinder. The gap between the stone discs was so adjusted as to pass the meal through 40-mesh sieve.

Table-1 *Physio-chemical characteristics of grains in wheat genotypes under uninfested and infested conditions*

		Genotypes						MEAN	LSD (p = 0.05)
		PBW 621	HD 2967	PBW 590	PBW 658	PBW 644	PBW 660		
Grain appearance score (Max. 10)	Uninfested	5.00±0.10	5.30±0.10	4.80±0.10	5.00±0.10	5.30±0.10	5.40±0.10	5.13	A= 0.08
	Infested	4.83±0.15	5.10±0.10	4.47±0.15	4.80±0.10	5.13±0.15	5.07±0.15	4.9	B = 0.14
Mean genotypes		4.91	5.2	4.63	4.9	5.21	5.23		AB = NS
Test Weight (Kg/hl)	Uninfested	71.71±3.31	72.53±3.02	75.04±2.34	74.00±2.29	73.57±4.53	73.37±2.30	73.73	A = 1.63
	Infested	69.42±2.13	70.17±1.53	70.46±1.49	70.17±1.04	70.57±0.90	69.75±0.66	70.09	B = NS
Mean genotypes		70.29	71.35	72.74	72.08	72.07	71.56		AB = NS
Grain hardness (Kg)	Uninfested	9.45±1.98	10.62±1.63	11.04±1.52	10.97±1.74	11.38±1.20	10.78±1.42	10.7	A = 0.75
	Infested	8.79±1.65	9.25±2.13	10.09±1.63	10.67±1.78	10.02±1.59	10.41±2.13	9.87	B = NS
Mean genotypes		9.12	9.93	10.56	10.82	10.7	10.59		AB = NS
SDS-Sedimentation Value (cc)	Uninfested	43.33±2.52	52.33±1.53	54.67±3.06	47.00±4.36	49.00±5.29	52.00±4.00	49.72	A = 2.34
	Infested	38.67±2.52	40.33±1.53	45.33±3.51	34.67±2.08	39.00±3.61	47.33±4.51	40.22	B = 4.06
Mean genotypes		41	46.33	50	40.83	42	49.66		AB = NS
Phenol reaction Score	Uninfested	3.87±0.21	3.20±0.20	3.83±0.40	3.60±0.30	3.73±0.25	3.60±0.20	3.64	A = 0.19
	Infested	3.90±0.40	3.73±0.25	4.00±0.30	3.83±0.25	3.80±0.20	3.90±0.30	3.86	B = NS
Mean genotypes		3.88	3.46	3.91	3.71	3.76	3.75		AB = NS

Values are mean ± SD. (A = treatments, B = genotype)

Table-2 *Plumpness of grains in wheat genotypes under uninfested and infested conditions*

		Genotypes							LSD (p = 0.05)
		PBW 621	HD 2967	PBW 590	PBW 658	PBW 644	PBW 660	MEAN	
2.8mm(g)	Uninfested	23.14±3.81	30.19±3.83	39.05±1.96	23.32±2.12	35.03±3.55	45.50±4.17	32.7	A = 2.38
	Infested	30.29±5.14	30.31±1.99	25.04±3.64	30.67±2.85	29.45±1.58	24.39±4.77	25.53	B= 4.13
MEAN		26.71	30.25	32.04	26.99	23.73	34.94		AB = 5.85
2.5mm(g)	Uninfested	51.27±2.99	43.02±3.68	40.61±3.14	50.89±3.47	50.77±3.27	39.00±3.42	45.93	A (?)= NS
	Infested	48.39±2.94	43.82±3.90	41.65±5.28	48.85±3.90	50.13±2.01	45.41±3.86	46.38	B = 4.24
MEAN		49.83	43.42	41.13	49.87	50.45	42.2		AB = NS
2.2mm(g)	Uninfested	18.05±2.30	22.45±2.23	16.12±3.35	22.68±3.27	11.95±2.54	12.86±3.01	17.35	A = 2.21
	Infested	25.17±3.61	19.15±3.38	31.52±3.88	19.54±3.51	19.36±3.93	24.07±3.07	24.8	B = NS
MEAN		21.6	20.79	23.82	21.11	20.65	18.46		AB = 5.42
Plup Kernels (g)	Uninfested	9.14±3.67	4.27±1.29	5.23±1.60	4.34±1.51	2.72±1.74	2.85±1.23	4.76	A = 1.40
	Infested	9.37±2.32	5.40±1.04	8.95±3.26	5.51±1.70	7.93±1.40	7.54±1.97	7.45	B = 2.44
MEAN		9.25	4.83	7.09	4.92	5.32	5.19		AB = NS

Values are mean ± SD. (A = treatments, B = genotype)

Table-3 *Gluten content and gluten index in wheat genotypes under uninfested and infested conditions*

			Genotypes						MEAN	LSD (p = 0.05)
			PBW 621	HD 2967	PBW 590	PBW 658	PBW 644	PBW 660		
Gluten content (%)	Dry	Uninfested	10.45±0.29	11.13±0.67	9.39±0.29	9.41±0.25	10.24±0.20	9.48±0.33	9.83	A = 0.25
		Infested	9.33±0.58	9.69±0.25	8.56±0.20	8.45±0.33	9.42±0.19	8.55±0.42	9.18	B = 0.43
		MEAN	9.88	10.41	8.97	8.92	9.83	9.01		AB = NS
	Wet	Uninfested	31.02±1.13	19.75±4.46	29.48±5.57	29.31±4.05	28.54±2.64	28.24±3.49	27.72	A = 2.08
		Infested	24.45±2.00	18.52±3.63	27.93±2.03	28.80±3.34	28.12±3.14	26.41±3.10	25.7	B = NS
		MEAN	27.73	26.58	28.7	29.05	28.33	27.32		AB = NS
Gluten Index		Uninfested	64.85±2.38	78.19±18.30	73.99±13.96	85.89±5.02	71.53±6.59	84.72±4.51	81.97	A = 5.97
		Infested	50.95±4.20	73.75±10.40	66.10±4.66	83.18±6.16	60.95±6.59	71.21±7.14	63.69	B = 10.34
		MEAN	57.9	75.96	70.05	84.53	66.24	77.96		AB = NS

Values are mean ± SD. (A = treatments, B = genotype)

Table-4 *Starch Pasting Characteristics in Wheat Genotypes Under Uninfested And Infested Conditions*

		Genotypes							LSD (p = 0.05)
		PBW 621	HD 2967	PBW 590	PBW 658	PBW 644	PBW 660	MEAN	
Pasting temp (ºC)	Uninfested	66.47±0.58	66.33±0.64	68.60±1.15	61.57±1.80	62.30±3.12	62.60±3.64	65.06	A = 1.26
	Infested	67.03±1.15	67.40±1.04	70.80±0.17	64.23±2.89	66.37±1.08	65.90±0.36	66.13	B = 2.18
	MEAN	66.75	66.86	69.7	62.9	64.33	64.25		AB = NS
Peak Viscosity (cp)	Uninfested	2281.67±153.73	2000.00±112.07	2343.00±80.55	1959.00±72.02	2324.00±148.70	2148.00±82.21	2175.94	A = 67.87
	Infested	1907.67±84.79	1810.00±50.41	1939.67±99.00	1789.00±47.00	1950.00±46.36	1883.67±127.11	1880	B = 117.55
	MEAN	2094.66	1905	2141.33	1874	2137	2015.83		AB = NS
Hold Viscosity (cp)	Uninfested	1274.67±55.52	1069.67±54.01	946.67±34.50	1381.00±28.69	1338.67±45.63	1148.33±48.09	1193.17	A = 40.22
	Infested	889.67±13.32	821.00±30.81	765.67±24.58	863.33±28.75	925.00±69.40	854.00±43.00	853.11	B = 69.67
	MEAN	1082.17	945.33	856.17	1125.16	1131.83	1001.16		AB = NS
Final viscosity (cp)	Uninfested	2739.67±50.01	2399.67±147.33	2225.00±58.28	2224.67±24.13	2902.00±43.21	2748.00±40.15	2517.61	A = 43.65
	Infested	2608.00±64.63	2337.67±77.84	2164.67±52.08	2187.33±32.59	2855.00±45.83	2594.67±23.35	2457.89	B = 75.61
	MEAN	2673.83	2368.66	2194.83	2206	2878.5	2671.33		AB = NS
Breakdown (cp)	Uninfested	1007.00±100.24	930.33±150.87	1012.33±82.03	962.00±91.93	985.33±194.30	999.67±121.08	982.78	A = 78.27
	Infested	1018.00±75.02	989.00±58.80	1076.33±102.40	1023.33±22.59	1025.00±64.97	1029.67±169.95	1026.89	B = 135.56
	MEAN	1012.5	959.5	1044.33	992.66	1005.16	1014.67		AB = NS
Setback (cp)	Uninfested	1465.00±103.12	1330.00±93.40	1278.00±12.17	844.00±86.47	1563.33±88.82	1599.67±77.09	1346.67	A = NS
	Infested	1930.00±43.31	1516.67±60.43	1421.67±37.11	1301.33±80.77	1718.33±77.94	1740.67±33.23	1604.78	B = 109.87
	MEAN	1697.5	1423.33	1351.33	1072.66	1640.83	1670.17		AB = NS

Values are mean ± SD. (A = treatments, B = genotype)

Table-5 *Response of tested genotypes against aphid complex*

Genotypes	Aphid Damage	Aphid scale	Category
PBW 621	14.6	IV	Susceptible
HD 2967	14.62	IV	Susceptible
PBW 590	17.29	IV	Susceptible
PBW 658	17.10	IV	Susceptible
PBW 644	17.46	V	Susceptible
PBW 660	17.24	V	Susceptible

**Aphid scale: 0: Immune, 1-5: Resistant, 6-10: Moderately resistant, 11-20: Susceptible, 21 and above: Highly susceptible*

The cleaned samples were conditioned to moisture of 15 percent and rested for about 40 hours before milling. Methodology for studying physical characteristics of grains: Grains were initially evaluated for various physical characteristics. Grain appearance score was determined subjectively out of a maximum score of ten giving due weight age to the grain size, shape, color and luster. Test weight was determined using the apparatus developed by the Directorate of Wheat Research, Karnal [12]. Weight of each grain was measured and expressed in Kg/hl. Grain hardness tester was used to determine the grain hardness by crushing ten grains one at a time selected randomly from the lot. Grain hardness tester was supplied by M/S Ogawa Seiki Co. Ltd., Japan. The average force in Kg required to crush each grain was recorded. Grain sorter (Sortimate, Germany) fitted with respective sized meshes was then used to analyse the grain plumpness. 100 gm of grains were separated according to the grain diameters (2.2mm, 2.5mm, 2.8mm and 2.8mm) and the grains of different diameters were then collected, weighed on an electronic balance and expressed in percentage. Methodology for studying chemical properties of grains: The phenol reaction of the wheat genotypes was determined by soaking 15-20 grains of each sample in the distilled water for 15-16 hours in petri plates. After that the water was drained off, 1 percent solution of phenol was added to the grains so that only three fourth of the grain was covered by the solution. The petri plates were covered and kept for 4 hours. After 4 hours the phenol solution was also drained off and the grains were dried of filter paper for 30 minutes. A subjective score (out of 10) was given with darker intensity of the colour. The SDS Sedimentation values of whole meal samples were determined by employing the method given by Axford *et al*. [13]. The gluten content and index values were evaluated using Glutomatic 2100 system supplied by M/S Perten, German. The instrument employs a 10g sample of whole meal using the AACC method. The wet gluten content and the dry gluten content were determined by following the procedure prescribed by the supplier. The gluten index was expressed as the percent wet gluten retained inside the centrifuge cassette. The whole meal pasting characteristics were evaluated for starch pasting characteristics on the Starch Master-Rapid visco-analyser (RVA) using 4.0 gm (14% moisture basis) of whole meal and 25.0 ml of distilled water in the canister.

Statistical Analysis: First of all, the data were tested for conformity to assumptions of analysis of variance (ANOVA) as dictated by tests of normality and homogeneity of variance [14]. Two-way factorial ANOVA was conducted with insecticide and genotypes as factors and physical and chemical characteristics as response variable. Means for response variables were compared using least significant difference (LSD) at 5 % probability level.

Results and Discussion

Physical characteristics of grains: Infestation by aphids resulted in significant reduction in grain appearance score (F = 33.93, d.f. = 1, 24, p<0.001) of infested grains (4.47-5.13) as compared to the uninfested grains (4.80 – 5.40) of all the test genotypes [Table-1] PBW 644 (3.20 %) showed minimum decrease in the grain appearance score whereas PBW 590 (6.87 %) showed maximum decrease as compared to their corresponding uninfested grain samples. Average values of timely sown rainfed genotypes showed maximum grain appearance score followed by timely sown irrigated and late sown irrigated genotypes. Karren [15] reported that infestation and damage caused by aphids result in flag leaves to curl and prevents the head from completely emerging. Thus, produces a "gooseneck" head that does not allow proper grain maturation. Infestation by aphids also 1-fold reduction in the overall test weight of the infested grain samples (69.42-70.57) as compared to the uninfested grain samples (71.71-75.04) of all the test genotypes (F = 17.09, d.f. = 1, 24, p<0.001). Test weight (TW) is an important predictor of milling yield for flour extraction rate for wheat. Critchley [16] reported that TW can be reduced by pest damage (78-92%). Hariri *et al*. [17] also observed 24% reduction in TW due to damage by pentatonic insects. Grain hardness is important for the flour industry because it has significant impacts on milling, baking and qualities of wheat [18]. Reduction in hardness of the infested grains (8.79-10.67) was observed as compared to the uninfested grains (9.45-11.38) all the test genotypes (F = 5.87, d.f. = 1, 24, p<0.05). Genotype PBW 658 was found to be the most tolerant variety, having maximum grain hardness score of 10.67 under the infested condition as compared to the other genotypes. Late sown irrigated varieties revealed peak grain hardness value followed by timely sown rainfed and timely sown irrigated genotypes. A continuous protein in hard wheat kernels physically traps starch granules and produces hardness [19]. Thus, lowering in the hardness of the grains after infestation in present investigations is difficult to explain. It might be due to other factors such as lowering in protein content, starch, gluten quality *etc*. Grain plumpness analysis helps in sorting the grains according to their size [Table-2]. Uninfested grains of all the genotypes were found to be maximum in 2.8 mm fraction of the grains, which subsequently decreased successively in 2.5 mm, 2.2 mm and plup kernel fraction. Very small amount of uninfested grains were seen in plup kernel fraction of the grains. However, on the other hand infested grain samples of all the genotypes increased successively from 2.8 mm fraction to plup kernel fraction with minimum amount seen in 2.8 mm fraction of the grains. Thus, it could be interpreted that the infestation by aphids resulted in poor development of the grains which resulted in matured grains having smaller diameter as compared to the uninfested normal grains. Chemical characteristics of grains: As a phloem-feeding insect, aphids reduce yield by sucking sap from wheat, which affects the grain filling stage [20]. Grain samples showed a significant decrease in SDS–sedimentation value after the infestation of aphids (F = 69.79, d.f. = 1, 24, p<0.001)). Higher the SDS sedimentation volume, the higher will be the strength of the protein. Thus, decrease in the SDS-sedimentation value in infested grains indicates lowering in the quality of proteins. PBW 658 experienced significantly higher reduction in the SDS-sedimentation values (26.23%) as compared to the other genotypes, which may be due to its higher susceptible nature. A decrease in wet and dry gluten was observed in current experiments [Table-3]. Even the gluten index was found to be significantly (F = 15.56, d.f. = 1, 24, p<0.05) lower in infested grain samples (50.95-83.18) as compared to the uninfested grain (64.85-85.89) in all the test genotypes. The highest decrease in the content of wet gluten was observed in PBW 621 (21.17 %) and in dry gluten it was observed in HD 2967 (12.93 %). This deterioration seems to be due to aphid infestation. Wheat bug (*Eurygaster* spp. and *Aelia* spp.) infestation alters the gluten status of wheat kernel, where wheat bug proteinase affects the disruption of gluten complex thus influencing the deterioration of rheological properties of wheat dough, poor baking performance and ultimately unsatisfactory final product appearance [17,21]. The degree of such deterioration depends on the intensity of wheat bug infestation and is often accompanied by significant economic damage [22-24]. The infestation of aphids increases overall phenol content of the grains as compared to the uninfested grains [Table-1]. PBW 590 was found to have the maximum phenol reaction score (4.00) under infested condition. Phenols are one of the most active groups of allelochemicals that unfavorably affect aphid growth, development and/or feeding behavior [25]. Thus, an increase in phenols indicates an increase in defense role of wheat plants. However, increase in phenolic compounds within the matured grains is considered undesirable in terms of quality characteristics of wheat grains. Singh *et al*. [26] during their study found that total phenols were relatively increased after herbivory feeding exclusively in endosperm of wheat making the grain hollow devoid of the content of the grains sparing the testa layer which is reported to contain polyphenols. High level of polyphenols restricts the bioavailability of macromolecules. All the pasting characteristics (pasting temperature, peak viscosity, hold viscosity, final viscosity, breakdown and setback) were significantly affected in aphid infested grains [Table-4]. Singh *et al*. [26] observed 12-64 percent starch loss at different levels of infestation by *R. dominca* and *T. granarium*. Infestation by aphids also reduced both the quality and quantity of the starch present in the wheat grains. Overall it could be concluded that aphids alters the overall quality parameters of the wheat grains.

Deteriorating effect of aphids was observed in the infested grains of all the genotypes; however, the severity of deterioration varied considerably among different genotypes. This might be due to difference in the duration and effectiveness of defence responses against aphid feeding in each genotype. The genotypes PBW 658 (Late sown irrigated), HD 2967 (Timely sown irrigated) and PBW 644 (timely sown rainfed) performed significantly better results than the corresponding genotype in each category. Late sown irrigated genotypes collectively were found to have better grain quality than timely sown irrigated and timely sown rainfed genotypes. Shortened grain filling time in late sown wheat could be a possible reason for better grain quality in late sown genotypes; however, a detailed study is required in this regard. Aphid feeding not only changes the biochemical content of wheat grains but also interferes with overall development and maturation of the grains, thus altering their physical characteristics (such as grain hardness, grain appearance and test weight) also. This may also lead to grains which may be completely not suitable for further processes and consumption. The information from the study will be useful for the breeders to develop aphid resistant wheat genotype. Since so far, all the released wheat varieties are susceptible against aphids [Table-5], therefore these less susceptible varieties under different sowing conditions could be utilized for further research to generate wheat varieties resistant against aphids. The tested quality parameter should be considered while developing aphid resistant cultivars to obtain nutritionally suitable wheat grains for human consumption.

Application of research: The study evaluates the various quality changes that are caused due to aphid infestation in different bread wheat varieties during the crop development. It reveals the various damages that aphid feeding causes on physical and chemical properties of wheat grain, making it unfit for various processes. The study also creates a paradigm for future studies focusing on plant protection against aphid feeding and generation of new resistance genotypes

Research Category: Aphid infestation

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