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Research Article

Effect of Temperature on Nutritive value on Wheat grain infested with *A. flavus*

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Abstract: Grain samples of five commonly grown wheat varieties viz.WH-542, PBW-343, UP-2003, Kundan and WH-502 were collected from three different storage centers i.e. Farmers house, Ware house and F.C.I Storage houses of Bihar state. Five samples of each commonly grown varieties were collected from different places and mixed well. Surface sterilized wheat grains were inoculated with 0.5 ml of *A .flavus* spore suspension ($2x10^{-2}$ spores/ml) of the respective fungal species. Inoculated wheat grains were kept in conical flask at different temperatures i.e. 10^0,20^0,30^0,40^0 and 50^0C for 7, 14, 21 and 28 days respectively. Wheat grains deterioration was studied based on biochemical qualities –Total Carbohydrate (Starch, Maltose), Protein (crude protein, gluten), Fiber (crude fiber) and Fats at 7 days interval up to 28 days at different temp. i.e. 10^0, 20^0, 30^0, 40^0 and 50^0C. Maximum deterioration of all the nutritive contents along with minerals was recorded as 23% after 28 days at 30^0C followed by 20^0 and 40^0C. Maximum deterioration was noticed at 30^0C after 28 days in Kundan and UP 2003 varieties followed by WH- 542, PBW-343 and WH-502. However, deterioration was observed as nil at 10^0 and 50^0C.
Keywords: Wheat grains, Nutritional quality, *Aspergillus flavus*, Grain deterioration.

INTRODUCTION:

Although, as many as 25 species of wheat have been recognized in the world, only three species of which namely*; T.aestivum / vulgare* Linn (Bread wheat), *T.durum* (Macaroni wheat) and *T.dicoccum* (Emmer wheat) are commercially grown in India. About 190 high yielding varieties of wheat have been released so far; however, their distribution depends on agro climatic condition of different zones. On the basis of yearly production presently India is considered as the 2nd largest producer of wheat after China and accounted for 12.06% share of total world wheat grains production. Bihar is potentially an important wheat growing state that contributes 5.7% towards national production of wheat growing area of the country with a low productivity of 1.9 tonnes/ha. Researches on biodeterioration of cereals, pulses and other agricultural commodities by mould and natural contamination of mycotoxins during storage have been advanced by several earlier investigators [1-4, 13] The detection to toxigenic moulds in cereal grain prior to their storage may help in the manipulation of storage practices to protect grain during storage and unhygienic condition. Several earlier workers including Ushamalini *et al*, [5] have reported the infestation of wheat grains by fungi during storage. The storage fungi/Moulds thrive on wheat grains by deriving nutrition thereby cause significant reduction in the weight and quality of grain. However, such biological event is not informally true for all varieties of wheat. Some of them were vulnerable to fungal association where as some varieties behave differentially in terms. Improper drying and storage under unhygienic condition make the grain vulnerable to microbial attack. The variable Indian climatic condition accompanied with heavy rains and increased level of relative humidity also stimulates the microbial activities on deterioration of quality of food value. During the journey of grain from field to storage and further effect of moulds under storage though depends on several factors but the genomic profile of the host also play important role moreover there is degree of variation of infection on different varieties, vis a vis loss in food value which might be due to variation in genomic sequence of wheat varieties. The occurance of these events in storage deteriorates the quality of wheat grain samples. The grain quality is greatly affected by prevailing environmental conditions by the time grains reaches physiological maturity from harvest. Grains storage is influenced by temperature and higher relative humidity which tends to deteriorate almost all kinds of different varieties of wheat grains rapidly. The problem becomes more serious and important because of periodic drought and other stress factors. Storage fungal activity can cause rapid deterioration of wheat grains, both in terms of dry matter and quality often loss of nutritive value with decrease variability. Biochemical changes leading to wheat grains deterioration, generally take place when the grains moisture level is favorable to

the growth of storage moulds. Colonization of storage fungi led to decrease in Carbohydrates, Protein, Fats & Fiber content in most cases [6]. Decrease in enzyme activity indicates loss of viability of grains. The scuteller amylase activity increase in un-aged grains while it decrease in aged seeds [7].

In present study, an attempt has been made to evaluate the extent of damage caused to the nutritional quality of different varieties of wheat grains viz., Total Carbohydrates (Starch, Maltose) Protein (Crude protein, gluten) Fiber (Crude fiber) & Fat activity of storage wheat grains due to the infested by *Aspergillus flavus.*

MATERIALS AND METHODS

Newly harvested five different varieties (WH-542, PBW-343, UP-2003, Kundan & WH-502) of wheat grains samples were collected from different collection centers (Farmer's houses, Ware houses & F.C.I Storage) where collected during the year 2010-2011 at different storage centers of Bihar, India. Biochemical and nutritional changes in the grains were studied infested by *Aspergillus flavus* for Total Carbohydrate (Starch, Maltose), Protein (crude protein, gluten), Fiber (crude fiber) and Fats.

Estimation of Starch

Starch content of wheat grain was determined by the procedure of Hedge *et al*., [8]. 100 mg of the sample was homogenized in hot 80% ethanol to obtain sugars. The solution was centrifuged and the supernatant was collected in test tube for starch estimation. The supernatant was then dried well over a water bath. In the dried sample added 5.0 ml of water and 6.5 ml of 52% perchloric acid and left for 20 min at 0°C. It was centrifuged and the supernatant was saved. The extraction was repeated by using fresh perchloric acid, Centrifuged and pooled the supernatants to make 100 ml. 0.1 ml of the supernatant was pipetted out and made up the volume to 1 ml with water. 4 ml of anthrone reagent to each tube was added and heated for eight minutes on a boiling water bath. It was cooled rapidly and the reading was taken at 630 nm in UV-Vis spectrophotometer and the glucose content was determined with the standard graph. Multiply the value by a factor 0.9 to arrive at the starch content.

Estimation of Maltose

Estimation of Maltose was determined by the procedure of Somogyi[9]. 100 mg of the samples was weighed and extracted the sugars with hot 80% ethanol twice. The supernatant was collected and evaporated it on a water bath at 80°C. 10 ml water was added and dissolves the sugars. 0.1 ml of the supernatant was pipetted out and made up the volume to 2 ml with water. 2ml distilled water was pipeted out in a separated tubes to set a blank. 1 ml of alkaline copper tartrate reagents was added to each tube. The tubes were placed in boiling water for 10 minutes. The tubes were cooled and 1 ml of arsenomolybolic acid reagent was added to all the tubes. The volume in each tube was made up to 10 ml with water. The absorbance was taken after 10 min at 620 nm in UV-Vis spectrophotometer. The amount of reducing sugars (maltose) present in the sample was calculated with following formula.

Absorbance corresponds to 0.1 ml of test = *x* mg of glucose

$$\textbf{10 ml contains } = \frac{\mathbf{x}}{\mathbf{0.1}}\mathbf{X10\ mg\ of\ glucose} = \%\ \textbf{of Maltose}$$

Estimation of crude protein

Estimination of crude protein was made by Microkjeldahl method (AOAC 1975) [10]. Each seed sample was powdered and 300mg of each sample was placed in 50-ml. Microkjeldahl flask and added with 60 mg catalyst and 7.5ml. H_2SO_4 the flasks were digested for 6-8h, after colling digest was diluted to 50ml in a volumetric flask and 5ml of liquid was introduced in Markhma's distillation unit through the side of funnel to which glass stopper was fitted. NH_3 librated was collected in 50ml conical flask containing 2% boric acid with an indicator and the distillate was titrated against 0.035 NH_3Cl_2 till end point was achieved. The crude protein was calculated as percent NX6.25 = crude protein.

Estimation of Gluten.

Gluten content was determined by employing the procedure of Paul [11] with slight modification. 10 gm sample of wheat flour is mixed with water to form dought and placed into the glutomatic washing chamber on top of the polyester screen. The sample was then hydrolysis with a 2% salt solution for 5 minutes then washed in running tap water in the washing chamber. The gluten content is then dried in hot air oven for 200^{o}c at 30 minutes and determined dry weight of the sample with the help of following formula.

$$\textit{Dry weight} = \frac{\textbf{100 } \textit{x dry weight of the gluten}}{\textbf{Weight of the sample}} \textbf{X} \frac{\textbf{100}}{\textbf{100 – Moisture content}}$$

Estimation of Crude Fibre

Crude Fibre content was determined by employing the procedure of Maynard *et al* [12]. 2 g of ground material was extracted with ether or petroleum ether to remove fat (Initial boiling temperature 35–38°C and final temperature 52°C). If fat content is below 1%, extraction may be omitted. After extraction with ether 2 gm of dried material was boiled with 200 ml of sulphuric acid for 30 min with bumping chips, Filtered through muslin and washed with boiling water until washings were no longer acidic and boiled with 200 ml of sodium hydroxide solution for 30 min. It was filtered through muslin cloth again and washed with 25 ml of

boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol. The residue was removed and transferred to ashing dish (preweighed dish W1). The residue was dried for 2 h at 130 ± 2°C, the dish was cooled in a desiccators and weighed (W2), left for 30 min at 600 ± 15°C. Cooled in desiccators and reweighed (W3).

% crude fibre in ground sample

$$= \frac{\text{Loss in weight on ignition (W2-W1) (W3-W1) X 100}}{\text{Weight of the sample}}$$

Estimation of Fat

Estimination of fat content A.O.A.C Leslic (Association of official Agricultural chemists) method was used is mentioned by Leslei Hart and Fisher [10]. An amount of 1gm of oven dried and powdered sample material were transferred to whatman no.1 filter paper with a porosity permitting rapid flow of paper with a porosity permitting rapid flow of petroleum ether 60-80˚C depending on the type of petroleum ether. The weight of the sample together with the dried whatman no.1 filter paper and the weight of the dry soxhlet flask were recorded the fat content of the samples were extracted using soxhlets extract for 12hrs. Using petroleum ether as solvent for fats. The ether was then removed from the mixture by cautions evaporation of the soxhlets flask in an oven at 100˚C for 30min., the extracted fat were left behind in the flask. The flask then cooled and weight again. The final weight of the sample and filter paper were also recorded again.

Fat content= (Initial weight of the sample and the filter paper)-(Final weight of the sample and filter paper).

RESULT AND DISCUSSION

Changes in Nutritional composition: - Maximum deterioration of all the nutritive contents along with minerals was recorded as 26% after 28 days at 30^0C followed by 20^0 and 40^0C. Maximum deterioration was noticed at 30^0C after 28 days in Kundan and UP 2003 varieties followed by WH- 542, PBW-343 and 502. However, deterioration was observed as nil at 10^0 and 50^0C.

Starch: Maximum deterioration was noticed at i.e. 11.58% after 28 days at 30^0C in Kundan varieties followed by WH- 542, PBW-343, UP-2003 and WH-502.(Tale-1 and Fig-1)

Maltose: Maximum deterioration was noticed at i.e. 1.42% after 28 days at 30^0C in UP-2003 varieties followed by WH- 542, PBW-343, Kundan and WH-502. (Tale-2 and Fig-2)

Crude protein: Maximum deterioration was noticed at i.e. 7.25% after 28 days at 30^0C in UP-2003 varieties followed by WH- 542, PBW-343, Kundan and WH-502. (Tale-3 and Fig-3)

Gluten: Maximum deterioration was noticed at i.e. 3.45% after 28 days at 30^0C in Kundan varieties followed by WH- 542, PBW-343, UP-2003 and WH-502. (Tale-4 and Fig-4)

Crude fiber: Maximum deterioration was noticed at i.e. 1.73% after 28 days at 30^0C in UP-2003 varieties followed by WH- 542, PBW-343, Kundan and WH-502. (Tale-5 and Fig-5)

Fats: Maximum deterioration was noticed at i.e. 0.82% after 28 days at 30^0C in UP-2003 varieties followed by WH- 542, PBW-343, Kundan and WH-502. (Tale-6 and Fig-6)

Table-1:-Showing changes in total Carbohydrate (Starch) contents in under storage.

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Temp (^{0}C)		**10˚C**	**20˚C**	**30˚C**	**40˚C**	**50˚C**
arieties	**Control**	**(±% change)**	**(±% change)**	**(± % change)**	**(± % change)**	**(±% change)**
WH-542	65.90%	65.90 (-0%)	59.75 (-6.15%)	57.05 (-8.85%)	58.06 (-7.84%)	65.90 (-0%)
PBW-343	64.94%	64.94 (-0%)	59.36 (-5.58%)	56.22 (-8.72%)	57.16 (-7.78%)	64.94 (-0%)
UP-2003	66.85%	66.85 (-0%)	59.35 (-7.50%)	57.05 (-9.80%)	57.88 (-8.97%)	66.85 (-0%)
Kundan	65.18%	65.18 (-0%)	58.20 (-6.98%)	53.60 (-11.58%)	55.36 (-9.82%)	65.18 (-0%)
WH-502	63.95%	63.95 (-0%)	57.20 (-6.75%)	54.45 (-9.50%)	55.80 (-8.15%)	63.95 (-0%)

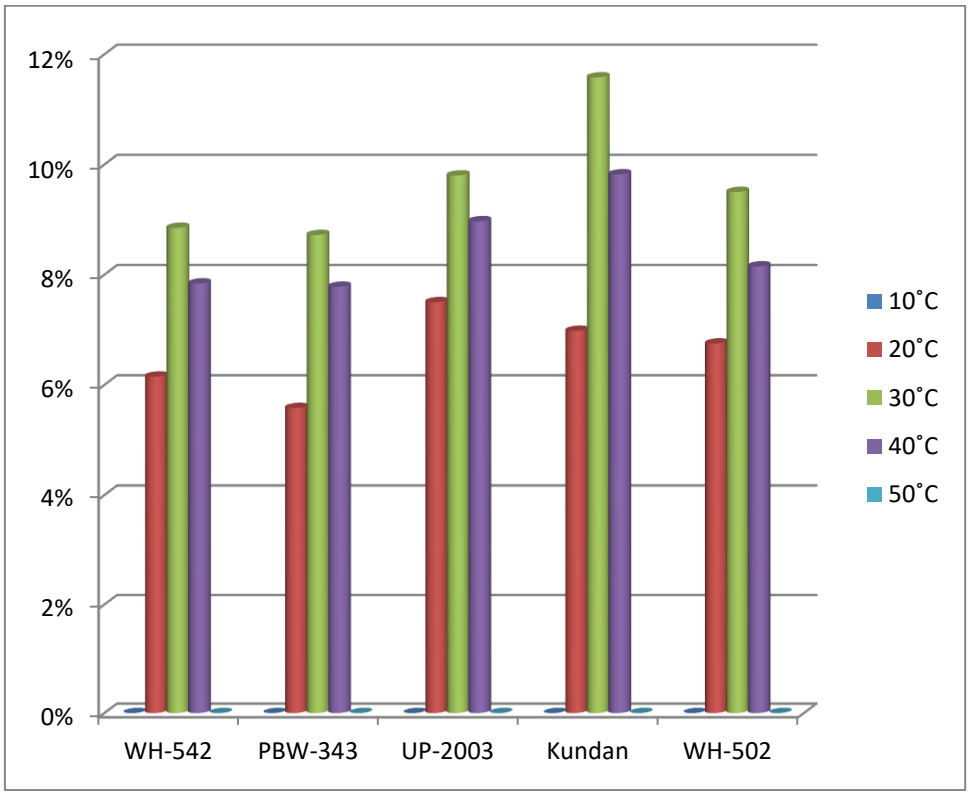

Fig-1: Showing changes in total Carbohydrate (Starch) contents in under storage

Table-2:-Showing changes in total Carbohydrate (Maltose) contents in under storage.

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Temp (^{0}C)		**10˚C (± % change)**	**20˚C (±% change)**	**30˚C (± % change)**	**40˚C (± % change)**	**50˚C (±% change)**
Varieties	**Control**					
WH-542	2.3%	2.3 (-0%)	1.16 (-1.14%)	0.98 (-1.32%)	1.08 (-1.22%)	2.3 (-0%)
PBW-343	2.1%	2.1 (-0%)	1.04 (-1.06%)	0.82 (-1.28%)	0.92 (-1.18%)	2.1 (-0%)
UP-2003	2.5%	2.5 (-0%)	1.2 (-1.3%)	1.08 (-1.42%)	1.15 (-1.35%)	2.5 (-0%)
Kundan	2.4%	2.4 (-0%)	1.16 (-1.24%)	1.02 (-1.38%)	1.12 (-1.28%)	2.4 (-0%)
WH-502	2.2%	2.2 (-0%)	1.2 (-1.0%)	0.94 (-1.26%)	1.02 (-1.18%)	2.2 (-0%)

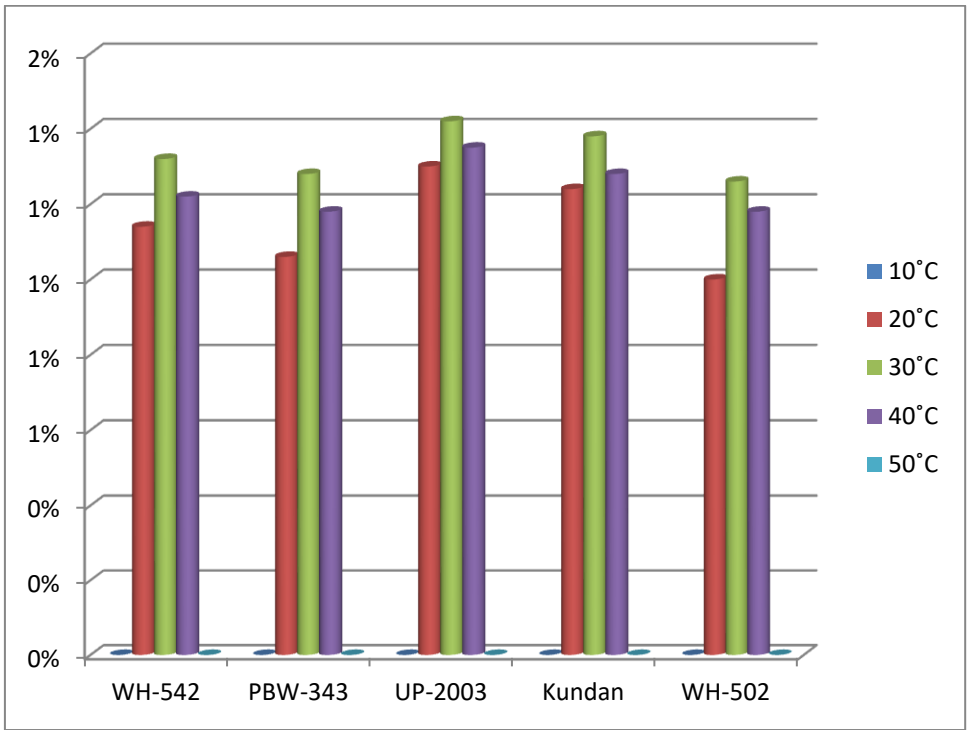

Fig-2: Showing changes in total Carbohydrate (Maltose) contents in under storage

Table-3:-Showing changes in Total Protein (Crude protein) contents in under storage.

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Temp (^{0}C)		**10˚C (± % change)**	**20˚C (±% change)**	**30˚C (± % change)**	**40˚C (± % change)**	**50˚C (±% change)**
Varieties	**Control**					
WH-542	16.8%	16.80 (-0%)	12.55 (-4.35%)	10.68 (-6.42%)	10.84 (-5.96%)	16.8 (-0%)
PBW-343	16.60%	16.60 (-0%)	12.94 (-3.78%)	10.64 (-5.98%)	11.82 (-4.92%)	16.60 (-0%)
UP-2003	17.15%	17.15 (-0%)	11.33 (-5.98%)	10 (-7.25%)	10.49 (-6.85%)	17.15 (-0%)
Kundan	16.75%	16.75 (-0%)	10.87 (-5.92%)	10.17 (-6.75%)	9.87 (-6.70%)	16.75 (-0%)
WH-502	16.18%	16.18 (-0%)	12.0 (-4.38%)	9.8 (-6.38%)	10.2 (-5.78%)	16.18 (-0%)

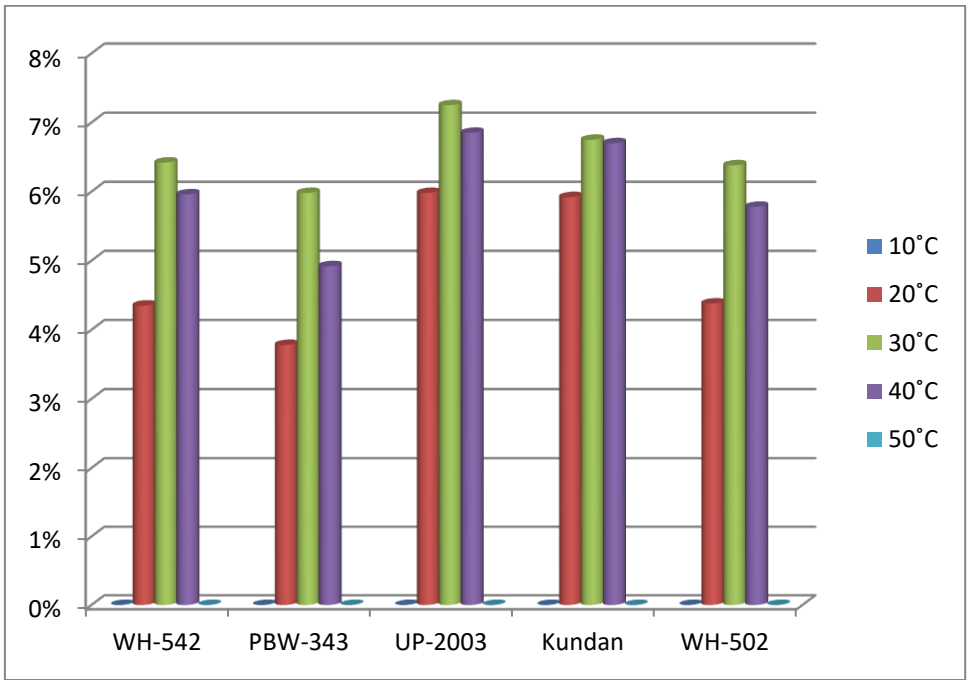

Fig-3:-Showing changes in Total Protein (Crude protein) contents in under storage

Table-4:-Showing changes in Total Protein (Gluten) contents in under storage.

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Temp (^{0}C)		10˚C (± % change)	20˚C (±% change)	30˚C (± % change)	40˚C (± % change)	50˚C (±% change)
Varieties	Control					
WH-542	7.59%	7.59 (-0%)	5.44 (-2.15%)	4.84 (-2.75%)	5.00 (-2.59%)	7.59 (-0%)
PBW-343	7.48%	7.48 (-0%)	5.50 (-1.98%)	4.75 (-2.73%)	5.0 (-2.48%)	7.48 (-0%)
UP-2003	8.02%	8.02 (-0%)	5.25 (-2.77%)	5.0 (-3.02%)	5.2 (-2.82%)	8.02 (-0%)
Kundan	7.95%	7.95 (-0%)	4.96 (-2.99%)	4.5 (-3.45%)	4.96 (-2.99%)	7.95 (-0%)
WH-502	7.38%	7.38 (-0%)	5.22 (-2.16%)	4.38 (-3.0%)	4.88 (-2.5%)	7.38 (-0%)

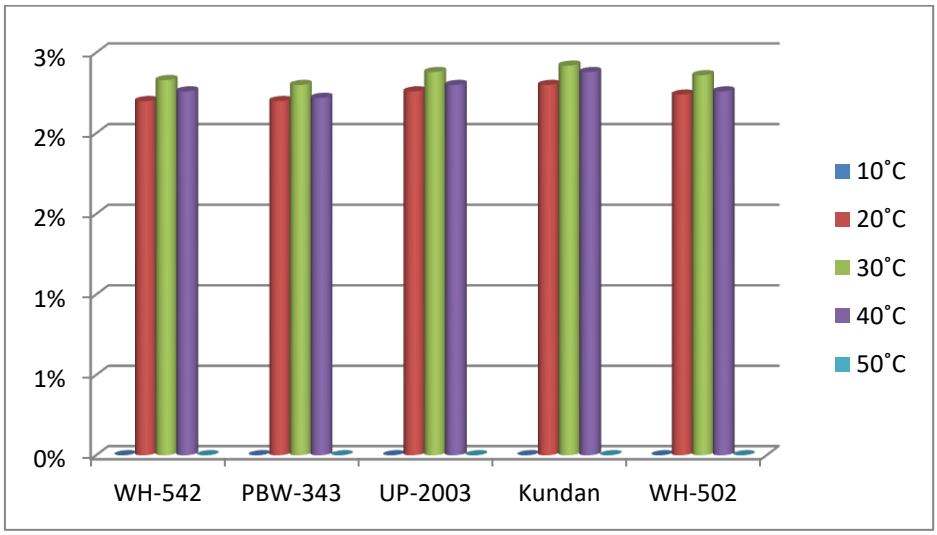

Fig-4:-Showing changes in Total Protein (Gluten) contents in under storage.

Table-5:-Showing changes in Total Fibre (Crude fibre) contents in under storage.

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Temp (^{0}C)		10˚C (± % change)	20˚C (±% change)	30˚C (± % change)	40˚C (± % change)	50˚C (±% change)
Varieties	Control					
WH-542	2.28%	2.28 (-0%)	1.28 (-1.0%)	0.78 (-1.5%)	0.90 (-1.38%)	2.28 (-0%)
PBW-343	2.32%	2.32 (-0%)	1.36 (-0.96%)	0.78 (-1.54%)	0.96 (-1.36%)	2.32 (-0%)
UP-2003	2.38%	2.38 (-0%)	0.9 (-1.48%)	0.65 (-1.73%)	0.90 (-1.48%)	2.38 (-0%)
Kundan	2.3%	2.3 (-0%)	0.92 (-1.38%)	0.65 (-1.65%)	0.86 (-1.44%)	2.3 (-0%)
WH-502	2.15%	2.15 (-0%)	1.16 (-0.98%)	0.70 (-1.45%)	0.82 (-1.33%)	2.15 (-0%)

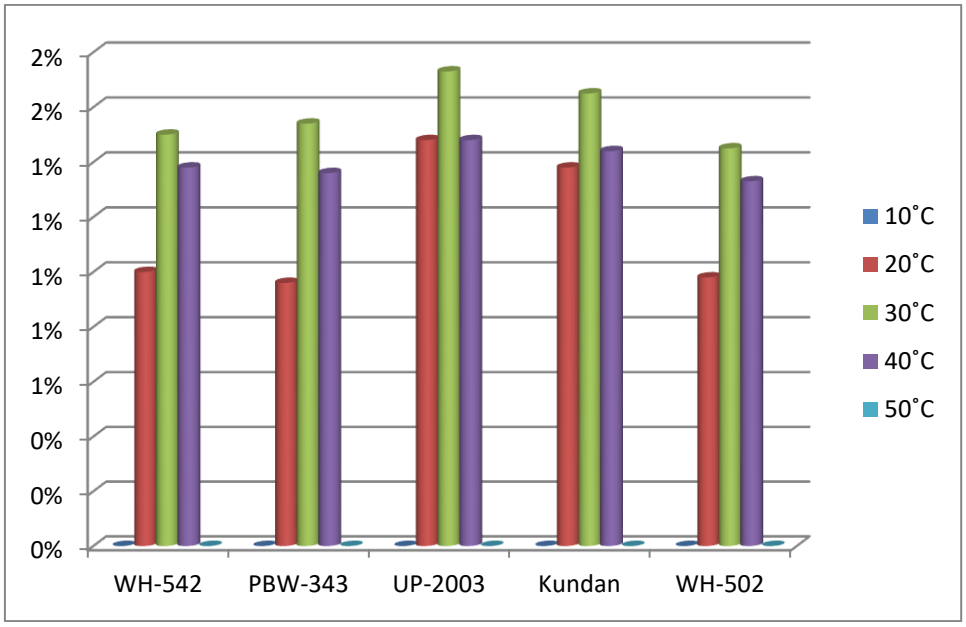

Fig-5:-Showing changes in Total Fibre (Crude fibre) contents in under storage.

Table-6:-Showing changes in Fat contents in under storage.

Percentage decrease over control due to *Aspergillus flavus*						
Incubation period after 28-days						
Temp (^{0}C)		10˚C (± % change)	20˚C (±% change)	30˚C (± % change)	40˚C (± % change)	50˚C (±% change)
Varieties	Control					
WH-542	1.25%	1.25 (-0%)	0.70 (-0.55%)	0.63 (-0.62%)	0.70 (-0.55%)	1.25 (-0%)
PBW-343	1.5%	1.5 (-0%)	0.9 (-0.60%)	0.82 (-0.68%)	0.88 (-0.62%)	1.5 (-0%)
UP-2003	1.6%	1.6 (-0%)	0.92 (-0.68%)	0.78 (-0.82%)	0.85 (-0.75%)	1.6 (-0%)
Kundan	1.4%	1.4 (-0%)	0.75 (-0.65%)	0.68 (-0.72%)	0.72 (-0.68%)	1.4 (-0%)
WH-502	1.15%	1.15 (-0%)	0.58 (-0.57%)	0.55 (-0.60%)	0.57 (-0.58%)	1.15 (-0%)

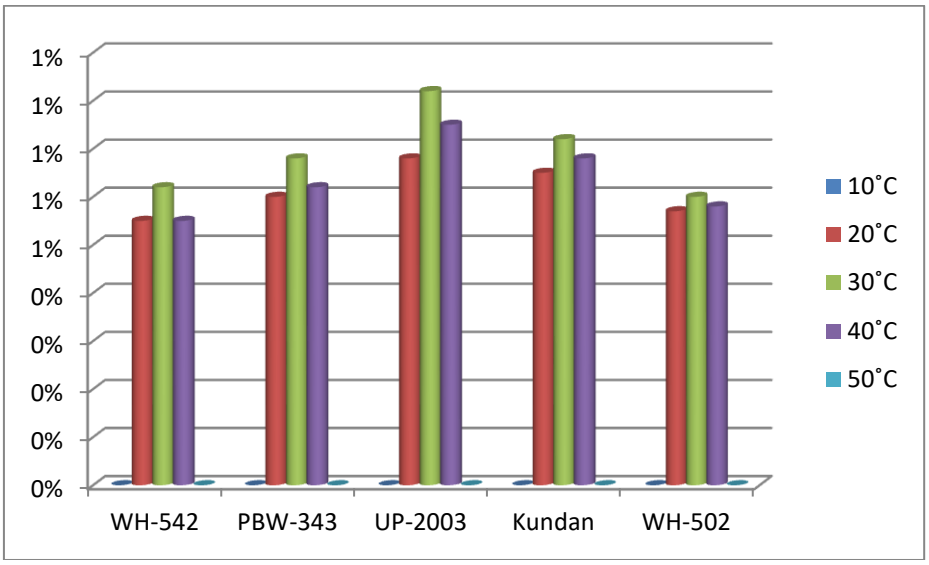

Fig-6:-Showing changes in Fat contents in under storage.

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