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RESEARCH PAPER

Effect of nanoparticles in volatile production during seed storage of groundnut

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Abstract : In the present study, seeds were evaluated for their influence at laboratory condition with dry dressing seed treatment, using inorganic and organic nanopowder that can maintain the seed quality in groundnut VRI 2. Fresh seeds of groundnut were treated with inorganic NPs of ZnO, Ag and TiO_2 each @ 750, 1000 and 1250 mg kg^{-1} and organic nanopowder of CLP and FSP each @ 1, 2 and 3 g kg^{-1} and stored for 12 months under ambient condition and to assess their effects on physiological parameter of seeds and explore of volatile metabolites emitted from the aged seeds relate with seed quality. Seeds treated with inorganic NPs and organic nanopowders did not vary for seed germination analyzed immediately after treatment. However, after 12 months of storage, seeds treated with ZnO NPs @ 1000 mg kg^{-1} enhanced germination (77%) against the control (66%). In the case of organic nanopowders, seed treated with FSP@ 2g and CLP@ 3g kg^{-1} had higher germination (75 and 75%) compared to control (66%). Volatiles emitted from the seeds treated with NPs revealed that the number of compound emitted under aldehyde, ketone, acids, ether, ester and carboxyl groups was less (20) while the control had 36 compouds, expressing a negative correlation to the germination. The present investigation clearly demonstrated the effect of inorganic NPs of ZnO @ 1000 mg kg^{-1} and Ag @ 1250 mg kg^{-1} and the organic nanopowders of FSP @ 2g kg^{-1} of seeds in maintaining the quality.

Key Words : Nanopowders, Groundnut, Seed quality, Volatile production

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) believed to be "King of oilseed crops", is native of Brazil (South America). Groundnut possessing high oil content (44-50%) and protein (25%) is a valuable source of vitamins E, K and B besides a richest plant source of thiamine and niacin, which are low in cereals. In India groundnut constitutes roughly about 50 per cent of the total oilseed production. The productivity level in India is very low mainly because about 80 per cent of the crop is grown under rainfed conditions with minimal inputs. In many parts of India groundnut seed is usually stored for a period of about 9 to 12 months before sowing. However, seed viability is getting lost quickly due to the production of free radicals by lipid peroxidaiton during storage (Murthy *et al.*, 2003). Several researchers reported that mid-term hydration-dehydration treatments performed better in improving germination and seedling vigour after storage in soybean (Mandal *et al.*, 2000) and okra (Kapri *et al.*,

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2003). Unfortunately, due to lack of improved post-harvest preservation technique, a large portion of seed yield gets lost in storage. However, it can be controlled to certain extent by adopting new technologies. Nanotechnology is an emerging technology and promises substantial help to agriculture, which can lead to a new revolution. Mixture of NPs *i.e.*, nano-SiO_2 and nano-TiO_2, increases nitrate reductase, also increase the seed germination and growth in soybean (Lu *et al.*, 2002). Natarajan and Sivasubramanian (2008) elucidated the role of NPs in seed viability and quality including nano-polymer for seed hardening, nano-sensors, nano-barcodes and use of magnetic NPs for aerial seeding. Senthilkumar (2011) and Sridhar (2012) further established the use of metal oxide NPs for improving germination upto 30 per cent in aged seeds of black gram and tomato. Botanicals act as catalysts for the production of reactive oxygen species (ROS) in a slow and sustained manner for maintenance of seed viability. Custard apple leaf powder and fenugreek seed powder possess appreciable antioxidants (Baskar *et al.*, 2007; Bukhari *et al.*, 2008 and Toppo *et al.*, 2009). Therefore, these botanicals which are potential source of natural antioxidants have been employed in seed treatments for vigour and viability maintenance.

Relationship between volatile aldehyde (VA) production during early germination and changes in seed vigour during natural as well as accelerated ageing (Harman *et al.*, 1982 and Wilson and McDonald, 1986) has been proved. The detection of VA during seed germination was suggested as a seed vigour test (Wilson and McDonald, 1986) and can quantify the incidence of weathering in soybean seed (Tyagi, 1992). Efforts to detect a volatile 'signature' for ageing that correlate with seed death have met with mixed results (Lee *et al.*, 2000b; Bicanic *et al.*, 2003; Tammela *et al.*, 2003 and Schwember and Bradford, 2005). For this work, it is designed to trap the volatiles released from the stored seed and the rate of volatile production will be analyzed through gas chromatography and mass spectroscopy (GCMS) and correlated with the duration of the asymptomatic phase of seed deterioration. Based on the nature of the detected compounds from the treated and control seeds, the seed quality will be assessed. The present investigation aims at understanding the interaction of NPs with groundnut kernels in improving the seed germination and assessing volatile aldehyde (VA) as a sensor of seed health.

MATERIAL AND METHODS

Seed treatment :

Groundnut cv. VRI 2 pods obtained from Oilseeds Research Station were shelled out and kernels were graded using round perforated metal sieves of 22/64" sieve and were dry dressed with synthesized nanoparticles of ZnO, Ag and TiO_2 @ 750, 1000 and 1250 mg kg^{-1} and organic nanopowder of CLP and FSP each @ 1, 2 and 3 g kg^{-1} using screw capped glass bottles at room temperature. The glass bottles containing seeds and nanoparticles were manually shaken gently for 3 min, 5 times at an interval of 3h. Seeds shaken without nanoparticles served as control. After dry dressing with the nano particles, the seeds were packed in cloth bag and stored under ambient conditions (25 ± 3^{o}C temperature and 95 ± 3% RH). Seed samples were drawn at monthly intervals up to 12 months and evaluated for the seed quality. Further, volatile metabolite profile was determined using GC-MS in order to establish a relationship with seed quality were carried out at the Department of Nano Science and Technology, TNAU, Coimbatore-03, during the period between 2013-2015.

Germination (%) (ISTA, 2010) :

The germination of the seeds was assessed with 50 kernels in three replications on sand medium. The test conditions of 25±2ºC and 95±3 per cent RH were maintained in a germination room. At the end of tenth day, the number of normal seedlings was counted and the mean was expressed as percentage.

Effect of test inorganic and organic nanoparticles on seed volatile emission :

Volatile compounds released by one year old NPs treated groundnut seeds were characterized using Thermo Scientific Trace GC Ultra chromatograph system (Thermo Fisher Scientific, Austria), coupled to thermo scientific DSQ II quadruple mass spectrometer. The compounds were separated using a 5 per cent phenyl methyl silicon fused-silica capillary column (TR-5 MS, 30m in length, 0.25 µm id., 0.25 µm film thickness). Groundnut seeds were preconditioned by placing them in between moist germination paper for 5 h at room temperature were incubated in a 20 ml headspace crimped vial at RT for 24 h. Air sample of 1ml was drawn from the headspace vial containing seeds at a standard depth of 25mm using a gas-tight syringe and injected directly into the GC-MS. Before drawing the sample, the vial was agitated for a minute in agitator at room temperature. The temperature of column was initially maintained at 45^0 C with a hold time of two minutes and then increased to 250^0C @ 10^oC per minute. The injector and detector were constantly maintained at temperature of 260^0 C and 270^0 C, respectively with a total run time of 32 min for good separation of the diverse compounds. Volatile compounds were identified by the fragmentation pattern of individual's compound and confirmed with the NIST (National Institute of Standards) Library database.

Statistical analysis :

The data obtained from different laboratory experiments were analyzed statistically by adopting techniques described by Panse and Sukhatme (1985). The critical differences (CD) were calculated at 5 per cent probability level. Wherever necessary, the percentage values were transformed into arcsine values. Volatiles emitted from the preconditioned seeds were correlated with germination as per the preconditioned seeds by Dewey and Lu (1959).

RESULTS AND DISCUSSION

In the current research, dry dressing of the groundnut seeds using three inorganic nanoparticles *viz.*, ZnO, Ag and TiO_2 @ 750, 1000 and 1250 mg kg^{-1} and and two organic nanopowder of CLP and FSP each @ 1, 2 and 3 g kg^{-1} concentrations each were done to assess its effect on seed quality parameter from initial to 12 months of storage at monthly intervals. Nanoparticle treated seeds significantly outperformed control in terms of germination and vigour index even after 12 months of storage. Significant differences were also observed between the nanoparticle treatments, storage period and their interaction. Among the tested NPs, ZnO @ 1000 mg kg^{-1} followed by Ag @ 1250mg kg^{-1} significantly increased the germination of treated fresh seeds, allowing for slow natural ageing upto 12 months (Table 1) compared

Table 1 : Effect of inorganic nanoparticles on germination (%) of fresh seeds of groundnut cv. VRI 2 under natural ageing

Treatments	Period of storage (p)													
	P_0	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	Mean
Control	91	89	86	81	75	68	64	61	57	52	49	44	42	66
	(72.54)	(70.63)	(68.02)	(64.15)	(60.00)	(55.55)	(53.13)	(51.35)	(49.02)	(46.14)	(44.42)	(41.55)	(40.39)	(54.33)
ZnO 750 mg	89	88	86	83	75	68	65	62	59	53	50	46	44	67
	(70.63)	(69.73)	(68.02)	(65.65)	(60.00)	(55.55)	(53.73)	(51.94)	(50.18)	(46.72)	(45.00)	(42.70)	(41.55)	(54.94)
ZnO 1000 mg	91	90	88	86	86	82	79	77	74	69	65	62	58	77
	(72.54)	(71.57)	(69.73)	(68.02)	(68.02)	(64.89)	(62.72)	(61.34)	(59.34)	(56.16)	(53.73)	(51.94)	(49.60)	(61.34)
ZnO 1250 mg	92	91	87	85	83	80	77	74	71	65	62	58	56	75
	(73.57)	(72.54)	(68.86)	(67.21)	(65.65)	(63.43)	(61.34)	(59.34)	(57.41)	(53.73)	(51.94)	(49.64)	(48.44)	(60.00)
Ag 750 mg	90	88	86	82	75	68	65	61	59	56	52	48	44	67
	(71.57)	(69.73)	(68.02)	(64.89)	(60.00)	(55.55)	(53.73)	(51.35)	(50.18)	(48.44)	(46.14)	(43.85)	(41.55)	(54.94)
Ag 1000 mg	91	89	87	85	82	78	75	68	66	63	59	57	55	73
	(72.54)	(70.63)	(68.86)	(67.21)	(64.89)	(62.02)	(60.00)	(55.55)	(54.33)	(52.53)	(50.18)	(49.02)	(47.87)	(58.69)
Ag 1250 mg	91	89	88	86	84	80	77	74	71	68	64	61	58	76
	(72.54)	(70.63)	(69.73)	(68.02)	(66.42)	(63.43)	(61.34)	(59.34)	(57.41)	(55.55)	(53.13)	(51.35)	(49.60)	(60.66)
TiO_2 750 mg	89	87	85	83	76	68	64	61	58	52	49	45	43	66
	(70.63)	(68.86)	(67.21)	(65.65)	(60.66)	(55.55)	(53.13)	(51.35)	(49.60)	(46.14)	(44.42)	(42.13)	(40.97)	(54.33)
$TiO_2$1000 mg	90	89	87	85	83	78	76	72	68	65	62	59	56	75
	(71.57)	(70.63)	(68.86)	(67.21)	(65.65)	(62.02)	(60.66)	(58.05)	(55.55)	(53.73)	(51.94)	(50.18)	(48.44)	(60.00)
$TiO_2$1250 mg	89	88	86	84	79	74	71	68	65	59	56	52	50	71
	(70.63)	(69.73)	(68.02)	(66.42)	(62.72)	(59.34)	(57.41)	(55.55)	(53.73)	(50.18)	(48.44)	(46.14)	(45.00)	(57.41)
Mean	90	89	87	84	80	74	71	68	65	60	57	53	51	71
	(71.57)	(70.63)	(68.86)	(66.42)	(63.43)	(59.34)	(57.41)	(55.55)	(53.73)	(50.76)	(49.02)	(46.72)	(45.57)	(57.41)

	Treatments	Period	Treatments × period
S.E. ±	0.78	0.89	2.83
C.D. (P=0.5)	1.55**	1.76**	5.58**

(Figures in parenthesis indicates arcsine values) ** indicate significance of value at P=0.01

to the remaining treatments including control. Prasad *et al*. (2012) also observed that ZnO nanoparticles at a concentration of 1000 ppm improved the germination, root growth, shoot growth, dry weight and pod yield in groundnut. In *Cicer arietinum*, Avinash *et al*. (2010) and Pandey *et al*. (2010) found that ZnO NPs increased the level of IAA in the roots (sprouts) and thereby an increase in the growth rate of plants.

Groundnut seed dry dressed with FSP nano powder @ 2 g kg^{-1} of seed followed by CLP nano powder @ 3g kg^{-1} and FSP nano powder @ 3 g kg^{-1} of seeds was found to be better than other treatments and control. The organic nanopowder (48%) treatment had increased germination percentage of naturally aged seeds by 12.5 per cent over control (42%). After a year of storage period, the highest germination per cent was evident in seed treated with FSP @ 2 g kg^{-1} (53%) and CLP @ 3g kg^{-1} (52%) followed by FSP @ 3g kg^{-1} (51%) (Table 2). The reason may be that is CLP and FSP possess appreciable level of antioxidant content (Baskar *et al.*, 2007; Bukhari *et al.*, 2008 and Toppo *et al.*, 2009). Botanicals act as a catalyst for production of reactive oxygen species (ROS) in a slow and sustained manner for maintenance of seed viability. The improvement in germination upon treating seeds with organic nanopowders might also be due to activation of cells resulting in enhanced of mitochondrial activity leading to the formation of more energy compounds and vital biomolecules which are made available during the early phase of germination as reported by Renugadevi *et al.* (2001) in rice**.** Similar results were reported by Renugadevi and Vijayageetha (2007) in cluster bean.

Effect of test inorganic and organic nanoparticles on seed volatile compounds emission :

A diverse array of volatile compounds is produced by seeds during storage suggesting that several reactions occur in seeds during deterioration process, some of which are detectable by volatile analysis (Hailstones and Smith, 1989; Zhang and Roos, 1997; Taylor *et al*., 1999 and Lee *et al*., 2000a). The identity of volatile compounds produced by seeds can indicate the kinds of chemical reactions that occur during storage and those reactions that might lead to loss of viability. The present investigation was made to find out the influence of NPs on volatile emission from 12 months stored groundnut seeds *viz*-a-*viz*., its quality. Seeds treated with inorganic and organic NPs had emitted fewer volatile compounds than the control. The GCMS analysis of the volatiles emitted from the NPs treated seeds collected in the head space revealed the presence of six major volatile compounds *viz*., aldehyde, ketoens, acids, ether, ester

Table 2 : Effect of organic nanopowders on germination (%) of fresh seeds of groundnut cv. VRI 2 under natural ageing

Treatments	Period of storage (p)													
	P_0	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	Mean
Control	91	89	86	81	75	68	64	61	57	52	49	44	42	66
	(72.54)	(70.63)	(68.02)	(64.15)	(60.00)	(55.55)	(53.13)	(51.35)	(49.02)	(46.14)	(44.42)	(41.55)	(40.39)	(54.33)
CLP 1g	92	90	87	83	76	72	66	63	58	53	50	44	42	67
	(73.57)	(71.57)	(68.86)	(65.65)	(60.66)	(58.05)	(54.33)	(52.53)	(49.60)	(46.72)	(45.00)	(41.55)	(40.39)	(54.94)
CLP 2g	89	88	86	84	82	79	73	70	65	62	58	52	49	72
	(70.63)	(69.73)	(68.02)	(66.42)	(64.89)	(62.72)	(58.69)	(56.79)	(53.73)	(51.94)	(49.60)	(46.14)	(44.42)	(58.05)
CLP 3g	90	89	87	85	83	81	76	74	69	65	63	55	52	75
	(71.57)	(70.63)	(68.86)	(67.21)	(65.65)	(64.15)	(60.66)	(59.34)	(56.16)	(53.73)	(52.53)	(47.87)	(46.14)	(60.00)
FSP 1g	89	87	85	83	76	73	67	64	58	55	52	46	43	68
	(70.63)	(68.86)	(67.21)	(65.65)	(60.66)	(58.69)	(54.94)	(53.13)	(49.60)	(47.87)	(46.14)	(42.70)	(40.97)	(55.55)
FSP 2g	89	89	87	87	84	82	77	74	71	66	63	57	53	75
	(70.63)	(70.63)	(68.86)	(68.86)	(66.42)	(64.89)	(61.34)	(59.34)	(57.41)	(54.33)	(52.53)	(49.02)	(46.72)	(60.00)
FSP 3g	91	89	87	85	83	80	75	72	69	64	62	54	51	74
	(72.54)	(70.63)	(68.86)	(67.21)	(65.65)	(63.43)	(60.00)	(58.05)	(56.16)	(53.13)	(51.94)	(47.29)	(45.57)	(59.34)
Mean	90	89	86	84	80	76	71	68	64	60	57	50	47	71
	(71.57)	(70.63)	(68.02)	(66.42)	(63.43)	(60.66)	(57.41)	(55.55)	(53.13)	(50.76)	(49.02)	(45.00)	(43.28)	(57.41)

	Treatments	Period	Treatments × period
S.E. ±	0.81	1.11	2.94
C.D. (P=0.5)	1.61**	1.19**	5.81**

Figures in parenthesis indicates arcsine values

** indicate significance of value at P=0.01

and carboxyl besides one or two unknown compounds. However, the control had seven unknown in addition to the above major six groups (Table 3). It is inconformity with the findings of Zhang *et al*. (1993 and 1995); Trawatha *et al.* (1995) and Mira *et al*. (2010) who reported on the release of acetaldehyde, ethanol and methanol as major volatile constituents of the seeds.

The number of volatiles emitted by the seeds treated with ZnO and Ag was relatively lower with 16.3 and 16.0, respectively than the control which had as much as 36 compounds. Among the organic NPs, FSP treated seeds released only 22.0 compounds while the CLP emitted 24.3 compounds (Fig. 1). In general seed treated with NPs resulted in the emission of reduced number of volatile compounds than the control. Upon seed treatment with NPs, the 12 aldehydes recovered in the control got reduced to 4.3, each 5 of ester and carboxyl to 3.5 and 3.1, respectively and 7 unknown to 1. However, NPs treatment resulted in emission of 1.5 number of ether, while control did not have. In contrast, ketone (3.2) compounds were more due to NPs treatment than control (2).

A significant of reduction in the number of compounds emitted due to NPs treatment was evident

Table 3 : Effect of inorganic and organic nanoparticles on the number of volatile compounds emission in aged groundnut seeds

Treatments	Aldehyde	Ketone	Acid	Ether	Ester	Carboxyl	Unknown	Total	% reduction over control
ZnO 750mg	6	2	1	2	3	6	1	21	
ZnO 1000	2	3	5	0	3	0	0	13	
ZnO 1250	3	3	4	0	3	2	0	15	
Mean	3.7	2.7	3.3	2.0	3.0	4.0	1.0	16.3	54.6
Ag 750 mg	7	3	4	1	2	2	2	21	
Ag 1000 mg	4	4	2	1	0	4	0	15	
Ag 1250mg	2	3	4	0	2	1	0	12	
Mean	4.3	3.3	3.3	1.0	2.0	2.3	2.0	16.0	55.6
TiO_2 750mg	5	4	6	1	6	1	0	23	
TiO_2 1000mg	4	3	7	1	5	1	0	21	
TiO_2 1250mg	4	4	4	2	3	4	0	21	
Mean	4.3	3.7	5.7	1.3	4.7	2.0	0	21.7	39.8
CLP 1g	8	5	6	0	4	3	0	26	
CLP 2g	5	3	7	3	3	5	0	26	
CLP 3g	4	2	4	2	4	4	1	21	
Mean	5.7	3.3	5.7	1.7	3.7	4.0	1.0	24.3	38.9
FSP 1g	4	2	4	1	2	7	2	22	
FSP 2g	4	3	7	2	4	1	0	21	
FSP 3g	2	4	7	1	7	2	0	23	
Mean	3.3	3.0	6.0	1.3	4.3	3.3	1.0	22.0	38.9
Control	12	2	5	0	5	5	7	36	
Over all means of NPs	4.3	3.2	4.8	1.5	3.5	3.1	1.0	20.1	

Table 4 : Correlation analysis of volatiles emission on germination in aged groundnut seeds

	Aldehydes	Ketons	Acid	Ether	Ester	Carboxyl	Unknown	Germination %
Aldehydes	1							
Ketons	-0.132	1						
Acids	-0.04	0.309	1					
Ether	-0.105	-0.172	0.047	1				
Ester	0.219	0.132	.578*	0.1	1			
Carboxyl	0.402	-0.401	-0.449	0.382	-0.101	1		
Unknown	.783**	-.529*	-0.135	-0.232	0.134	0.431	1	
Germination %	-.762**	-0.019	0.189	-0.009	-0.22	-.506*	-.513*	1

* = Correlation is significant at the 0.05 level (2-tailed) ** = Correlation is significant at the 0.01 level (2-tailed)

which had 55 per cent reduction in case of both the ZnO and Ag while 39 per cent in the case of TiO_2 and organic nanopowders. Inorganic nanoparticles except TiO_2 resulted in low amount of volatile, while organic nanopowder produced relatively high amount of volatiles. Production of volatile compounds in seeds singnal to the deterioration process. The amount of volatiles increased with increasing storage period and temperature of storage which suggested that these volatiles were produced metabolically (Zhang *et al.*, 1993).

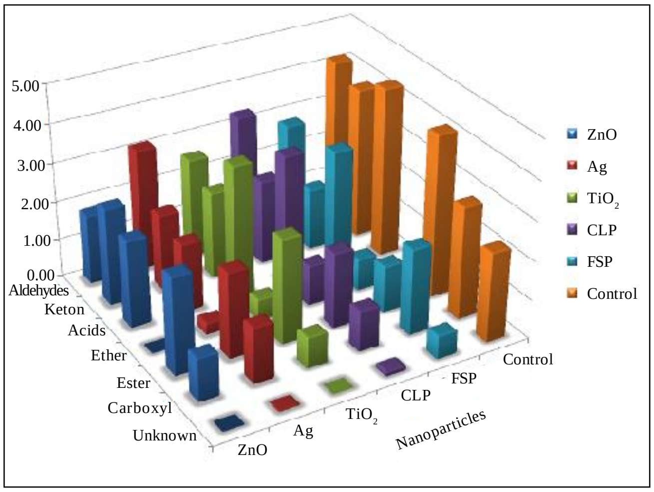

Fig. 1 : Effect of inorganic and organic nanoparticles on the number of volatile compounds emission in aged groundnut seeds

Area of the peak in the graphical output of GCMS analysis is indicative of the strength of the compound. Hence, the per cent area of all the peaks expressed by the each compound was also considered for analysis, so as to identify the strength of the six major volatiles. The highest peak area of 4.4 per cent was due to acid groups while the least was with carboxyl (2.3 %). The strength of the unknown compound in the control was 2.3 per cent while due to NPs 0.18 per cent. The total strength of volatiles produced by the seeds without treatment NPs was 21.9 per cent, while the least was with ZnO treatment (10.5 %) accounting for 52 per cent reduction over control (Fig. 2). There was no variation between TiO_2 and FSP (12.3 % in the % area). The least reduction of 40.7 per cent over control in the per cent area was observed in seeds treated with CLP. Among the organic NPs, FSP treated seed resulted in the least area than the CLP.

Considering the number of compounds and per cent area of the volatiles though there was a positive relationship in case of control as well as CLP, there was mismatch to the number and strength of the other tested NPs. Though Ag ranked the least in release of number of compounds, as for as area is concerned ZnO only exhibited the least per cent area. The germination level of the seeds treated with the test NPs and the volatile compounds were correlated (Table 4).

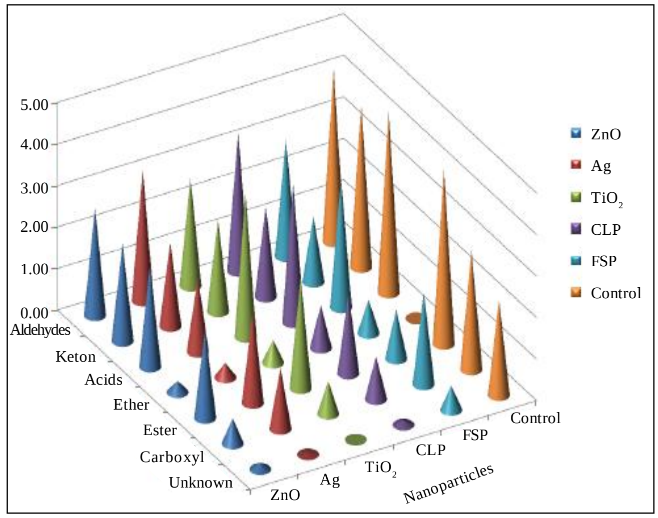

Fig. 2 : Effect of inorganic and organic nanoparticles on the strength of volatile compounds emission in aged groundnut seeds

The correlation study revealed that per cent germination of seeds treated with NPs had lower amount of aldehydes, carboxyl and unknown volatile compounds revealing the significant negative correlation among them. Efforts made to detect a volatile compound for assessing the ageing or to pinpoint specific substrates, products, or reaction pathways that can correlate with seed death have met with mixed results (Wilson and McDonald, 1986; Hailstones and Smith 1989; Lee *et al*., 2000b and Schwember and Bradford, 2005).

Different types of volatile molecules produced as a result of peroxidation can act as an indicator molecule for detecting seed viability. Volatile compound produced in preconditioned seeds ready for germination when treated with NPs whether inorganic and organic were less compared to control.

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