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Influence on oilseeds and legume seed physiology under insect pest and pathogenic infestation

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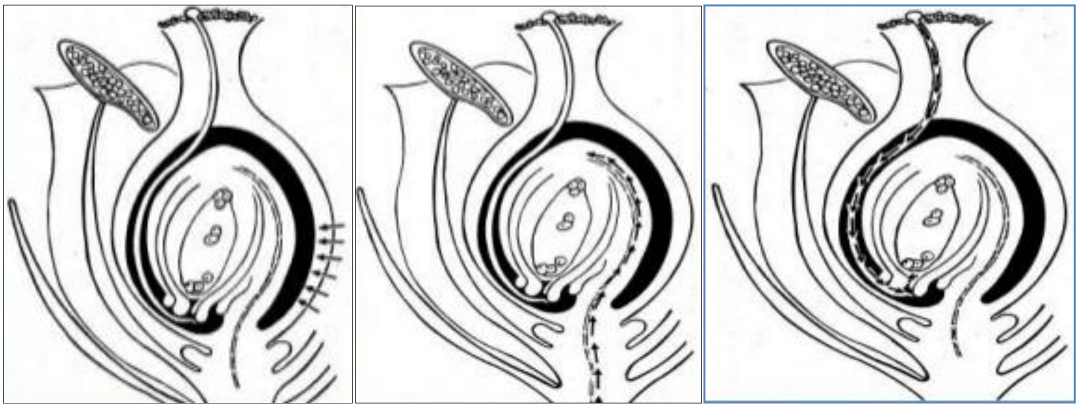
Abstract
Seed quality is the most important parameter for a successful harvest. The most detrimental factor is seed health refers to the presence or absence of any diseases or insects in the seed lot. Rapid deterioration in seed quality occurs due to insects and pathogens both in standing crop as well as storage causing reduction in viability and vigourness. The infestation induced deterioration in quality can be related with various physiological process changes at cellular, metabolic and biochemical level *viz*., chromosomal aberrations, impairment of DNA, erroneous RNA synthesis, protein synthesis breakdown, lipid peroxidation, membrane disruption and free radical damage, that causes several detrimental effects on seed. Seed deteoriation is undesirable for crop production.

Keywords: Seed quality, physiological change, insect and disease, biochemical reaction

Introduction
An excellent and profitable crop yield requires a superior crop stand. In order to achieve that healthy seed is the base, which means it should be free from any diseases. Infected seed serves as the primary source of inoculums for the spread of diseases. At the times the infection is secondary as it infects the plant in field but gradually moves to the developing seeds. Plants face several abiotic and biotic stress challenges, which not only lead to yield reduction but also the quality of produce, particularly in legumes and oilseeds. The extent of crop loss due to weeds account 37 per cent while insects and diseases account about 29 and 22 per cent respectively (Yaduraju, 2006) [25]. A wide range of physio-chemical changes takes place due to the impact of these pests whereas few of them improvise the protection level. Accumulation of phyto-chemicals through physiological, morphological, and chemical changes is the vital change in plants. Various biochemical constituents present in the cells and tissues of the host plant also exert a profound influence on biology of pathogens and insects. Oilseed and legumes play an important role in agriculture and industrial economy. According to feeding habitat, pest is divided into 2 groups- chewing type and sucking type mouth parts. Mostly the fungi attacking the crop plants broadly belong to 25 different species (Duan *et al*., 2007) [9], but *Aspergillus, Penicillum and Fusarium* species are to blame for the most part yield loss. The infections also reduced cooking and nutritive quality, along with production of undesirable odours and colour, and decrease germination and vigour of developing seeds (Quenton *et al*., 2003 and Castillo *et al*., 2004) [20, 7]. In addition, they produce mycotoxins those are health hazard for man and animals, make products unacceptable for edible purposes or lower their market grade. The fungal infestation on seed coat causes reduction in seed viability producing abnormal seedlings (Selcuk *et al.*, 2008) [23]**.** The diseases can be broadly classified into three categories *viz.*, bacterial, fungal and viral. Majority fungal species infect developing seeds while it is still attached to the mother plant (Neergaard, 1979; Agarwal and Sinclair, 1993) [18, 2]. The pathogens can affect the seed by directly infesting the seeds, establishing themselves on emerged seedling or surviving on the commercial processes involved in crop production. The infection can be active (*i.e.* found within the seed) or passive (*i.e.* found on the surface of the seed or as a contaminant in the seed lot) (Agarwal and Sinclair, 1993) [2]. The active seed infection has three common routes *i.e.* a) penetration through ovary wall, b) systemic infestation via the vascular system and c) penetration through the floral parts (Fig. 1). Through the active infection the direct connection between embryonic and endospermic tissue becomes disconnected as seed develops and the potential for transmission of infection is affected by the degree of internal system. The indirect systemic infection moves through the stigma or flower and fruit to the embryo. So, management of pest problems in these crops could increase the quality and quantity of their product.



(a) (b) (c)

Fig 1: Routes of active seed infection: (a) penetration through ovary wall, (b) systemic infection *via* vascular system and (c) penetration through floral parts.

Infestation and Response

1. Insect -pest Infestation and biochemical responses in oilseed and pulses

The chewing type insects mostly feed on the different plant parts *i.e.*, flowers, foliage, stem, roots or buds. The Flea beetle eat partial parts of leaves, making hole on leaf surface, while weevils caused bud abscission, reducing yield of pods, defoliation, delayed flowering.

Borer infestation causes tunnel into stem, roots and pods, that's why reducing leaf area and photosynthetic rate. There is no proper development of seed due to less amount of photosynthates for seed production (references??). These borers generally affect the seeds by feeding on the seed constituents thus leading to deformed and poor quality seeds. The sucking type insect *viz,* aphids, bugs, thrips, white fly, mites and jassids cause discoloration or twist and curl leaves. The plant may discolor from tiny yellow speckles (spider mites), larger darkened spots (bugs), reducing the canopy area of plant and therefore its photosynthetic capacity reduced. Aphids and white fly suck cell sap and also cause coating of black sooty mold growth on honey dew deposits or transmitting viruses. The sucking pests affect the seeds more than the chewing ones.

The seeds attacking insects mostly belong to Coleoptera (beetles) and Lepidoptera (moths and butterflies) order. The lepidopteran larvae entangle through silky secretion and turn developing seeds into entwined lumps whereas; the coleopterans, larvae as well as adults feed on the crop and cause the damage. The insect attacks may be primary, i.e. able to attack intact grains or secondary, attacking already previously damaged seeds.

In a recent study of Khan *et. al.*, (2015) [3] it was revealed that aphid attack in *Brassica* species in 1st and 2nd week of february, causes severe deterioration of leaves and heavy losses to crops by forming large colonies on leaves, stem and inflorescence. Aphid infested plant show slow growth because it secretes cell sap, which resulted seed loss of 9-77%. It also causes 11% reduction in seed oil content. In another study by Elamin *et al.* (2015) [1] in sesame crop shows the impact of sesame seed bug, with five levels of infestation *i.e.* 0, 15, 30, 45 and 60 days revealed that the highest crop loss was obtained with 60 days of infestation, the weight loss reached on an average of 13% while the loss of oil content was almost 50% of the control. The free fatty acids like oleic acid, reached an average of 20%, also the other fatty acids were affected. The injured seed shrivelled and when the damage was severe, the sesame seeds which contains about 50% oil of its control, remained completely empty and it is the also reason of its bitter taste. The fatty acids methyl esters were also significantly decreased and there was loss of 4-12% in fatty acids within two months of infestation by the sesame seed bug. Rani (2005) [21] reported that total protein and reducing sugar were comparatively lower in pods, and seeds in the tolerant varieties with lower pod borer damage than in the susceptible pigeon pea varieties. Bommesha *et al*. (2012) [6] also reported that, the low content of protein, reducing sugar, total sugars and high phenol content offers resistance against insect pest incidence in pigeon pea.

Apart of this, the groundnut seed has common infestation of sucking bugs. Anna *et al.,* (2013) [16] found that when groundnut seeds were left on the field unattended from 3- 35 days before it was decorticated noticed that if the pods were left on the field for more days, the infestation of groundnut sucking bug (*R. littoralis)* is quite high that goes upto 90% loss in oil content. Most of the major and minor pests infested during the vegetative to pre-maturity stages (45-95 DAS) and the maximum infestation occurred during pod formation and pod filling stages (50-80 DAS) of the crop. The major pests attacking groundnut are hairy caterpillar (*Spilarctia obliqua*), common cutworm (*Spodoptera litura F.*), jassid (*Empoasca terminalis*), leaf miner (*Stomopteryx nerteria* M.) and leaf roller (*Anersia ephippias* M.) (Biswas, 2014) [5]. One of the prominent plant responses to insect herbivore attack is the induction and accumulation of oxidative enzymes, such peroxidase and catalase triggered due to damage to the cell wall. These enzymes, because of their potential roles in plant signaling, synthesis of defense compounds, and/or in oxidative stress tolerance, has been implicated in plant resistance to insect herbivores. Taggar *et al.* (2012) [24] on a study in black gram crop indicated that in general, whitefly infestation increased the activities of peroxidase and decreased the catalase activity. Resistant genotypes NDU 5-7 and KU 99-20 recorded higher peroxidase and catalase activities at 30 and 50 DAS under whitefly-stress conditions as compared with non-stressed plants.

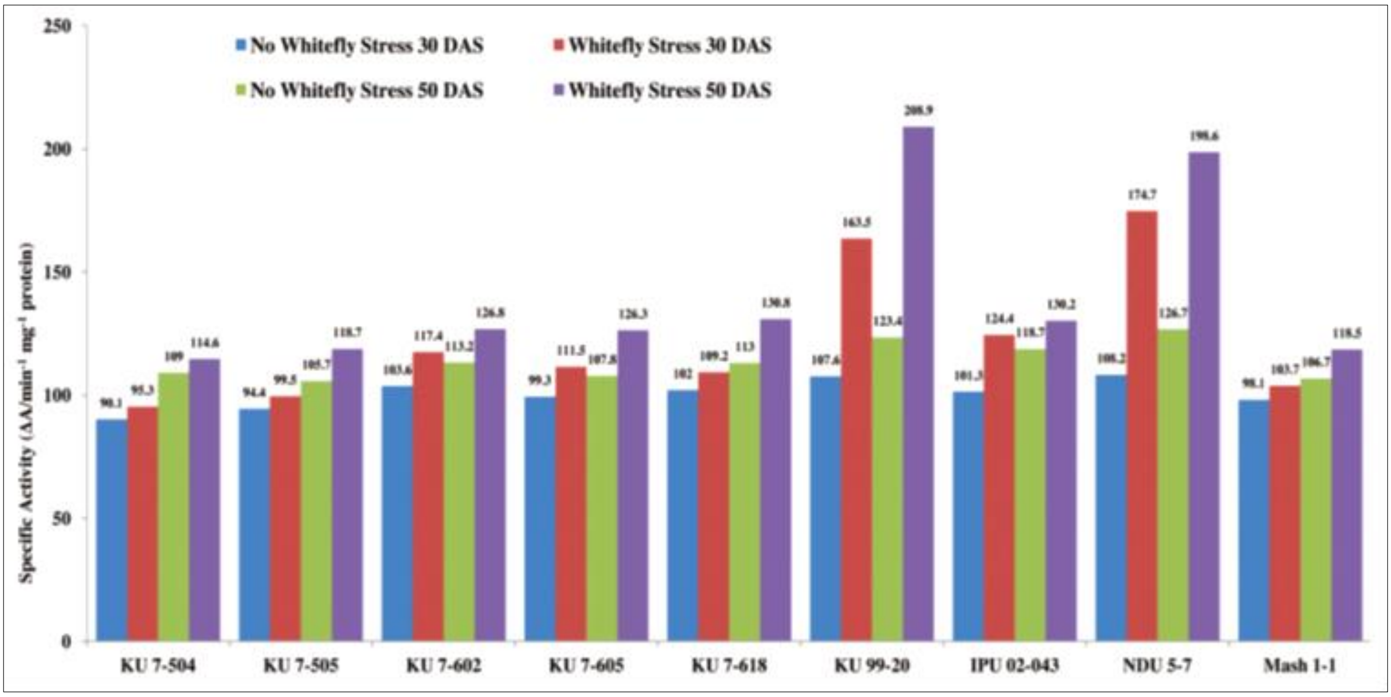


Fig 1: Specific Peroxidase activity (30 and 50 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding (Taggar *et al.*, 2012) [24]

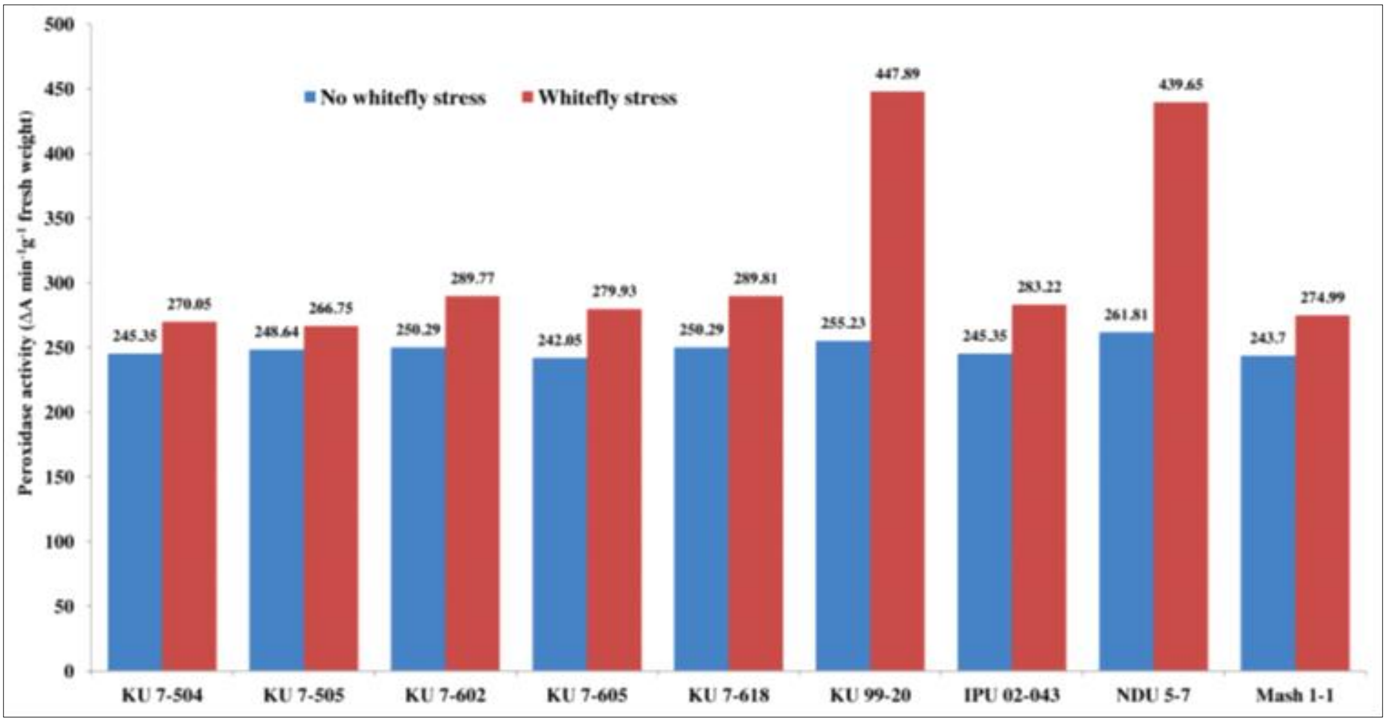


Fig 2: Peroxidase activity (30 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Adopted from Taggar *et al. (*2012) [24].

Santos *et al.* (2016) [22] reported that during storage, the presence of damage in the dry pods increased the percentage of seeds attacked by *Corcyra cephalonica* and was significantly negatively correlated with normal seedlings (−0.70), with the seedling emergence in sand (−0.57) and with the accelerated aging (−0.64) results but was significantly positively correlated with dead seeds, directly interfering with seed quality. However, the percentage of seeds damaged by *Cyrtomenus mirabilis* did not change greatly after storage because infestation by this insect occurs in the field. The correlation between *C. cephalonica* and *C. mirabilis* infestations was positive and highly significant (0.80). This indicated that both insects favoured the increase in attack of the other in their presence.

2. Pathogen infestation and biochemical changes in legumes and oilseed

The major pathogen studies by Sidddique (1976) concluded that out of the three types of pathogenic infestation, the fungi played highly impactful role in deteriorating the seed quality. Kandhare (2016) [13] reported the following to be the common dominant seed borne fungi which are affecting the pulses *i.e. Alternaria tenuis, A. alternate, Aspergillus flavus, A. niger, A. carbonarius, A. fumigates, A. nidulans, Cladosporium spp., Colletotrichum truncatum, Chaetomium globosum, Curvularia lunata, Dreshlera tetramera, Fusarium moniliforme, F. oxysporum, Penicillium spp., Rhizopus stolonifer and Macrophomina phaseolina.*

Similarly, the dominant species of bacteria found are *Pseudomonas spp., Xanthomonas spp. and Rhizoctonia spp.* The predominant species affecting soybean seeds at 28° C are *Aspergillus sp.*, followed by Penicillum sp. At 45° C *A. fumigatus* was the predominant species whereas *Penicillium* spp. one of the most abundant genera at 28° C but absent at 45° C. The infection due to virus was more indirect type, *i.e*., they affected the crop plant due to attack of their transmitters and later affected the seed while its development.

The seed borne fungi of pathogenic nature are found to cause a severe reduction in the germination ability and emergence, plant health, number of plants giving yield and lastly the economic yield *i.e.,* seed (Czyzewska, 1987) [8]. Upon infection there is an increase in the catabolic activities in the cell. This is slow in low temperature and humidity conditions. There has been found to be a swift augment in the moisture content in the seeds infected by microbes and this rise in moisture content contributes to an increase in the catabolism of the seed. The high humid and temperature conditions enhance this effect. The biochemical composition of the seeds was rigorously affected by infection showing a sharp deplete in the food reserves. The protein compounds undergo rapid oxidation thereby causing the functional proteins and enzymes to loss their functional property (Agarwal and Sinclair, 1993). Dwarka and Rajasab (2016) [2, 10] reported that the common dominant fungi infecting Pongamia seeds were found to be *Colletotrichum cladosporoids, Alternaria sp.* and *Aspergillus sp.*, thereby causing a reduction in lipid, reducing sugar, carbohydrates and oil by 0.21, 0.44, 2.74 and 5 per cent respectively (Table 2). There is peroxidation of the lipid also proceeds to a smaller extent in the contaminated legume cell. Initially there is an increase in the activity of α-amylase, catalase and peroxidase which caused the breakdown of the primary metabolites. Later, there was a decline in their activity. There is a reduction in the activity of protease, lipase, catalase, ribonuclease, acid phosphatase, amylase, DNA se and dehydrogenase enzymes (Mahjabin *et al.*, 2015) [15]. The breakdown of primary metabolites increased the content of free amino acids and aldehyde compounds, which get accumulated. The activity of lipases from endogenous fungi were lesser than the exogenous infection. When there is a chemical reaction happening there are several intermediates formed in the process, but if any one of the intermediates get accumulated in the cell beyond the limit that is required for the completion of the reaction then the process gets hindered. Enhanced activity of these enzymes scavenges the ROS and provides bioprotection in tolerant species but reduced activity seen in susceptible species. Reduced activity of peroxidises and catalases caused a rapid oxidative stress, as they are scavengers of the free radicals in plan cells, which is favoured by a high humidity and temperature (Mahjabin *et al.*, 2015) [15]. There is production of ROS like hydrogen peroxide, superoxide radicals, singlet oxygen which upon absence of the appropriate scavenging enzymes supersedes the toxic limits putting oxidative stress on the crop. They affect the membrane structures of various cell organelles along with cell itself. The impairment of the enzymes hampers the production of the enzymes which can breakdown these ROS. Infected seeds showed a decline in sugar as well as protein content, which is due to the utilization of these components by the microbes infecting in their growth (Aziz and Mahrous 2004) [4]. Infection by various fungal pathogen leads to seed deterioration oriation by causing various cellular, chemical as well as metabolic alterations. This is due to impaired transcription and faulty enzyme synthesis. The RNA impairment affects the activity of ribosomes to dissociate as a result of which the protein synthesis also gets hindered. The loss of elasticity of protein and cellular matrix ultimately cause folding of the DNA structure. They can lead to chromosomal aberrations damaging the DNA structure. Changes in the protein, reducing and non-reducing sugars were observed in cowpea seeds infected with either *A. nidulants* and *A. tereus* (Maheshwari and Mathur, 1987) [14]. Chemical composition (protein, lipid, carbohydrate, crude fibre) of sesame and soybean seeds were influenced by *A. flavus* growth (Farag, 1990) [12]. Invasion of seeds by some pathogens may result in biochemical deterioration and change in quality of seed nutrient as infected in soybean seed with A. flavus (Agarwal and Sinclair, 1993) [2]. The biochemical changes lead to lower levels of ATP and sugar content, inactivation of synthesizing enzymes, activation of hydrolytic enzymes, reduced gaseous exchange and accumulation of toxic compounds. The increase in free amino acids and radicals lead to membrane disruption. There is a sharp decrease in the oligosaccharide content, which are linked to stability of membrane. The infections can cause a molecular oxidation of the various compounds leading to break down of the same into reactive carbonyl and nitrogen groups, which later diffuse into the cell and are degraded into the advanced glycolysis end products. This causes a loss of the membrane integrity due to break down in its original structural compounds (Agarwal and Sinclair, 1993) [2]. Lipid peroxidation also causes damage to cell membrane and its constituents due to destruction of lipid structure. It also damages the lipid bilayer of mitochondria there by impairing the growth respiration, thus reducing the synthesis of ATP, which is the energy currency for growth of seed. The integrity of the soybean seed coat may also influences pathogen development. As soybean seed considered fragile in nature, Hill and West (1982) also found that naturally occurring pores on the soybean seed coat surface provide a mean for fungal hyphae to penetrate the palisade layer eventually culminating invasion of the hourglass layer of the seed coat. The damage to membrane by infection is due to auto oxidation of poly unsaturated fatty acids. This causes removal of leachates or constituents from cell. Thus there is an increase in electrical conductivity of surrounding solution and this is directly correlated to the intensity of membrane damage and the intensity of fungal infection. Protein, total and reducing sugar contents decreased gradually with increase in the RH values (Lokesh and Hiremath, 1993). Embaby *et al.* (2013) [17, 11] reported that *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *Fusarium oxysporum*, *F. moniliforme*, *Penicillium sp.* and *Sclerotia sclerotium* to be the most commonly infecting the seeds of soybean followed by pea and beans. *A. parasiticus and F. moniliforme* produce mycotoxins afflatoxin and fumosin beyond the permitted levels depending on the degree of infection. In bean *A. parasiticus* and *F. moniliforme* produced 196.58 mg aflatoxin and 198 mg fumosin per kg of seeds respectively, which was seen inthe HPLC chromatograph in isolates from infected samples. These mycotoxins altered the chemical composition of the developing seeds with the highest decrease in carbohydrate and fat content in all three crop species. The various microbes upon infection also interfered in the normal plant functioning by the production of mycotoxins, which are the secondary metabolites produced by the fungi like ochratoxin, fumosin, aflatoxin, get accumulated in the seeds causing a reduction in its quality as these toxins if consumed beyond the permitted level can prove to be cancerous. The mycotoxin isolated from

Aspergillus fumigatus when applied on seeds of two varieties of seeds at gradually increasing concentrations showed a decline in the germination percent (Table 3). The highest reduction in germination by about 84.8 % was found at the concentration of 500 mg L^{-1} (Noura, 2014) [19]. From this, we can conclude that mycotoxins from these pathogenic fungi also cause a demur in the vigour parameters which in turn shrink in the germination rate. There is a rapid increase in the respiration of the plant but the photosynthetic activity gets a setback due to this the net photosynthesis in reduced. The maintenance respiration is a enhanced as the plant responds to the injuries caused by the infection in order to repair them. All these changes affect the vigour parameters like germination rate, root and shoot growth, seed vigour as well as vitality. In the same manner, accumulation of free fatty acids and loss of germinability accompanied by mould growth also reported. Loss of viability is due to the chemical and structural changes which are irreversible. *Aspergillus flavus, A. niger* and *Fusarium moniliforme* caused a reduction in protein content in Redgram, whereas, seed germination and vigour was reduced in green gram and black gram due to *Aspergillus flavus* and *Fusarium semitectum* (Kandhare, 2016) [13] (Table 4). The analyses of the effect of the incidence of microorganisms on peanut seed germination and vigor showed negative and highly significant correlations between the *Aspergillus spp*. fungi and the results of the tests on germination (−0.82), accelerated aging (−0.82) and seedling emergence in sand (−0.85) (Santos *et al.*, 2016) [22].

Conclusion

Most of the losses that occurred in crop cultivation has been reported by pest and pathogens (about more than 50%).The deterioration in the quality of seeds was mostly due to fungi attack which accounted around 57% and that of bacteria was around 14% which was induced by primary fungal attacks. The viral infections in seeds directly were accounted negligible but infections reported up to 8.69%. Among the various groups of insects that causes maximum damage, borers were most prominent which affected seed quality not only during the storage but also when the seeds were intact with mother plant. The infection was triggered by presence of high humidity, temperature and moisture which are conducive for growth of microbes especially fungi, which play an important role in deterioration of seed quality and viability. The maximum deterioration in seed quality was found in terms of poor oil quality and reduced protein content in the oil seeds and pulses, respectively. The quality parameters are the upshot of the several interlinked physiological and biochemical processes *viz*, respiration, photosynthesis, DNA and chromosome structure, cell wall and membrane stability, carbohydrate, protein and lipid synthesis, which are easily altered by changes in the enzyme activity, chromosomal aberrations, breakdown in primary metabolite synthesis, amount free amino acid, glycerol and reduced sugars and free radical damages. The pest and pathogen attack bring about a change in this existing plant physiology and biochemistry which is the major cause of significant yield and economic loss. Proper efficient and timely management and regulation of these pest and pathogen attack is the key to a successful crop. More focus needed on effect of these infections at various stages timely so that the plant biochemical and molecular level could be understand at operational stages and minimal infestation can be managed without compromising seed quality constituents.

Table 1: Effect of common dominant fungi on protein content of black gram, green gram, gram and red gram (Kandhare 2016) [13].

Common dominant fungi infestation	**Total protein content in seed (mg/g of seed)**			
	Black gram	**Green gram**	**Gram**	**Red gram**
Control	140	136	168	173
Aspergillus flavus	120	121	107	136
A. fumigatus	*136*	*130*	*158*	*159*
A. niger	123	124	146	153
Dreschlera tetramera	136	122	153	160
Fusarium moniliforme	100	120	104	125
Rhizopus stolonifer	131	133	162	170

Table 2: Estimation of Primary Metabolites in Healthy and Infected Seeds of *Pongamia Pinnata* (Dwarka and Rajasab 2016) [10].

Metabolites	**In Healthy**	**In Infected**	**% Loss**
Total Lipid	0.79mg/gm	0.58mg/gm	0.21%
Protein	2.66mg/gm	1.95mg/gm	0.71%
Reducing Sugar	1.26mg/gm	0.819mg/gm	0.44%
Carbohydrate	15.44mg/gm	12.70mg/gm	2.74%
Starch	24.70mg/gm	18.2mg/gm	6.5%
Extraction of Oil	37ml/100gm	32ml/100gm	5%

Table 3: Effect of fungal metabolic toxin from *A. fumigatus* on the germination rates of chick pea (Noura, 2014) [19]

Concentration (mg L^{-1})	**Control**	**3.5**	**15**	**30**	**125**	**250**	**500**
Germination (%)							
ILC 32-79	99	92	64	63	18	17	16
FLIP 85-54	99	94	84	74	18	17	15

Table 4: Effect of *Fusarium moniliforme* and *Aspergillus parasiticus* on the chemical composition (Embaby *et al.*, 2013) [11].

Seed Crops	**Beans**				**Pea**				**Soybean**			
Chemical Composition	H	I	L%	R%	H	I	L%	R%	H	I	L%	R%
Protein												
Fusarium	31.1	26.8	4.3	13.8	32.2	22.7	9.5	29.5	45.0	40.9	4.1	9.1
Aspergillus	31.1	22.4	8.7	27.9	32.2	30.2	2	6.2	45.0	41.6	3.4	7.5
Carbohydrate												
Fusarium	38.7	21.0	17.7	43.7	30.9	17.3	13.5	43.9	30.7	25.2	5.5	17.9
Aspergillus	38.7	18.3	20.3	52.6	30.9	12.8	18.0	58.3	30.7	24.6	6.1	19.8
Fat												
Fusarium	21.9	2.7	19.2	87.6	3.9	2.9	1.09	27.3	22.8	16.5	6.3	27.8
Aspergillus	21.9	1.5	20.4	93.1	3.99	1.7	2.29	57.3	22.8	10.9	11.9	52.1
Ash												
Fusarium	7.2	5.1	2.1	29.1	5.0	4.2	0.8	16	6.1	5.3	0.8	13.1
Aspergillus	7.2	3.4	3.8	52.7	5.0	2.3	2.7	54	6.1	1.36	4.74	77.7
Moisture												
Fusarium	8.49	47.2	38.7	82	8.43	52.3	43.87	83.8	7.77	30.1	22.4	74.4
Aspergillus	8.49	45.9	37.4	81.4	8.43	61.3	52.7	85.9	7.7	36.5	28.8	78.9

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