BIOCHEMICAL AND NUTRIENT STATUS IN CHICKPEA DUE TO POWDERY MILDEW (*Leveillula taurica* (Lev.) Arnaud)

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By

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CERTIFICATE

This is to certify that the thesis entitled "BIOCHEMICAL AND NUTRIENT STATUS IN CHICKPEA DUE TO POWDERY MILDEW (*Leveillula taurica* (Lev.) Arnaud)" submitted by Mr. SUNIL S. ANGADI for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY to the University of Agricultural Sciences, Dharwad is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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1. INTRODUCTION

Pulse established an excelled integrant of Indian agriculture as they are the paramount source of protein in predominantly vegetarian diet. They form almost an essential component of Indian diet as dal-roti denoting complete and satisfying food. Among the commonly used food items of our daily meal, pulses supply highest amount of protein. Besides, pulse supply nutritious fodder, feed, fuel and they specially fortify soil through biological nitrogen fixation (BNF) which is economically sound and environmentally acceptable and thereby they sustain the productivity of the cropping system.

Chickpea (*Cicer arietinum* L.), also known as bengal gram, kadale and channa. It is the only widely cultivated species of the genus *Cicer* and belongs to the subfamily faboidae of the fabaceae family. It is the most important pulse crop in India. The crop has multiple use in rural as well as in urban India. The seeds are consumed in various forms both raw and processed has a part of daily meal or as a fast food and it supplies high protein (17.5-27.9 %) through seeds. The leaves, young tender shoots and immature seeds are used as green vegetables, which have some medicinal value besides blood purifier. The husk, broken shoots and leaves left after threshing and milling are used as cattle feed. In short, the people of India utilize every part of almost every stage of the crop.

Chickpea (*Cicer arietinum* L), is the third most important pulse crop after bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) on a world basis, but of first importance in the Mediterranean basin and South Asia. Chickpea is grown in more than thirty three countries. Ninety two per cent of the area and eighty nine per cent of the production of grain is covered by semi-arid tropical countries. It is the second most important pulse in terms of area under cultivation throughout the world after dry beans, but ranks third in terms of production, following dry beans and peas.

The origin of chickpea is thought to have been in the area of present-day south eastern Turkey and neighbouring northern Syria. It has since spread to many other geographical regions of the world because of its ability to grow in diverse environments. It was first grown in Turkey around 7,500 B.C. *Cicer arietinum*, the cultivated species of *Cicer*, has been domesticated from *C. reticulatum* Ladizinsky. a closely related wild species. After its domestication in Middle East this crop progressed further throughout the Mediterranean region, India and Ethiopia (Ladizinsky, 1975).

It is a hardy, deep-tap rooted, dryland crop sown on marginal lands, which can grow to full maturity in conditions that would be unsuitable for most crops. The deep-tap root system enhances its capacity to withstand drought conditions. It is usually well suited for cultivation in cooler areas with low rainfall. The yield is maximal when the legume crop is grown in sandy-loam soils possessing an appropriate drainage system; since it is very sensitive to excess water availability. Also, very cold conditions can greatly reduce the productivity of chickpea. It is basically a *rabi* crop, grown in months of September-November and harvested in the months of February- April. Maturity period ranges from 95-110 days after sowing.

There are two main commercial types of chickpea, the “Desi” type chickpea is grown in the semi-arid tropics with smaller size and dark coloured seeds which may vary from yellow to black and having a thicker seed coat while the “Kabuli” type is grown in temperate regions with big size, smooth and light coloured seeds having a thinner seed coat (Muehlbauer and Singh, 1987). The Desi type chickpea contributes to around 80 % and the Kabuli type around 20 % of the total production.

It is rich and most available source of protein both for human and animals. Chickpea seed contains 18–30 per cent protein, nearly 40 per cent carbohydrates and 3-6 per cent oil (Gil *et al*., 1996) and moreover, it is a very good source of many kinds of minerals such as calcium, phosphorus, magnesium, iron, zinc, potassium and manganese (Ibrikci *et al*., 2003).

Chickpea is most widely cultivated as winter crop throughout India, especially in northern states. All over the world, chickpea occupies an area of 12.0 m ha with a production of 10.9 mt and productivity of 913 kg/ ha (Anon., 2013). India stands first in area and production of chickpea followed by Pakistan and Turkey. In India chickpea is cultivated over an area of 10.2 m ha and with a production of 9.5 mt and average productivity level of 967 kg/ha. Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Uttar Pradesh, Andhra Pradesh and Karnataka are the major and leading chickpea growing states sharing over 95 per cent area. In Karnataka, chickpea is grown in an area of 0.92 m ha with a production of 0.57 mt with productivity of 622 kg/ha (Anon., 2014).

Chickpea yield affected by many biotic and abiotic stresses. Among abiotic stresses, chick pea is severally affected by drought stress since the crop is mostly grown during *rabi* season.

Among the biotic stresses, it is affected by about 67 fungi, 22 viruses, 3 bacteria and 80 nematodes. (Nene *et al*., 1996) but among these pathogens only few cause economically important diseases (Haware, 1998). But India alone has been reported and recorded highest number of pathogens rising to 89 pathogens in 1995 from 35 in 1978 (Nene *et al*., 1996).

The fungal diseases affecting chickpea are wilt caused by *Fusarium oxysporum* Schlechtend.Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, ascochyta blight caused by *Ascochyta rabiei*, leaf spot (*Alternaria* sp.), rust *(Uromyces ciceris-arientini),* gray mould (*Botrytis cinera*), powdery mildew *(Leveillula taurica)*, *Pythium debaryanum, P. ultimum*, dry root rot *(Rhizoctonia bataticola* and *R. solani*) foot rot (*Sclerotium rolfsii* and *Sclerotinia sclerotiorum)* and wilt *(Verticillium albo-atrum*).

Chickpea powdery mildew is getting greater attention in recent years. It was not a problem in northern parts of Karnataka till 2010, and then onwards this disease spreading very fast in alarming phase as may be one of the threat for the cultivation of the crop in near future. It is caused by *Leveillula taurica*, powdery mildew epidemic begins late in the season so yield usually less affected by the infection. However, early infection of pathogen leads to more yield loss.

In India, powdery mildew (*Leveillula taurica*) on chickpea was reported first by Mandhare *et al*. (2005) from Maharashtra. In Washington state, the disease appeared late in the growing season of October 2007, and the disease was found on chickpea fields in an experimental farm near Pullman, Whitman Co., Washington (Attanayake *et al*., 2008).

Typical powdery mildew symptoms and signs characterized by a white powdery growth on the leaves, stems and pods. The initial symptoms consist of tiny slightly discolored spots on the upper surface of leaves. Theses spots enlarge and become covered with powdery fungal growth. The tissue beneath affected areas may turn purple and later brown. If infection is severe, affected plants turn brown and die. Affected seeds become brown (Attanayake *et al*., 2008). Warm days and cool nights favour disease development. It causes remarkable damage and yield loss.

Appearance of powdery mildew on chickpea may be due to changing climatic conditions. Since it is fungal disease influenced by many weather factors as well as biochemical composition of plant alters the disease incidence and to know the effect of powdery mildew incidence on biochemical and nutrient status in infected plants. Considering these points the following objectives were formulated in the proposed investigation.

1. To study the changes in the levels of chlorophyll, phenols and sugars.
2. To study the changes in the proteins and amino acids.
3. To study the quantitative status of nutrients due to pathogenicity.
4. *In vivo* evaluation of fungicides.

2. REVIEW OF LITERATURE

Chickpea is an important pulse crop grown throughout the world which is affected by various diseases. Among them powdery mildew caused by *Leveillula taurica* (Lev.) Arnaud is an emerging disease. Although lot of literature available on powdery mildew of other pulses, cereals and vegetables, but not much work has been done on powdery mildew of chick pea. Therefore, it is considered necessary to review the literature of *L. taurica* on chick pea and also on other related crops.

2.1 Changes in the levels of chlorophyll, phenols and sugars

2.1.1 Chlorophyll

Photosynthesis is a process of synthesis of chemical compounds with the aid of solar radiant energy. It involves a series of reactions that converts radiant solar energy into stable chemical energy. In the process photosynthesis, it involves certain leaf pigments *viz.,* Chlorophyll, xanthophyll and carotenes. Among these Chlorophyll is the major leaf pigment which actively involves in photosynthesis.

Chlorophyll pigments are universally present in all photosynthetic tissue plants. Chlorophyll 'a' and chlorophyll 'b' are present in higher plants. The total chlorophyll pigment get altered and affected by the process of disease development which ultimately leads to biochemical changes in the plant tissue.

Ashtaputre (2005) who conducted biochemical parameters of healthy and diseased leaves of susceptible (Byadgi dabbi) and moderately resistant (Taiwan-8) varieties as influenced by powdery mildew of chilli caused by *Leveillula taurica.* In Taiwan-8 higher amount of chlorophyll a, b and total chlorophyll (21.4, 4.8, 26.2 mg/g and 11.6, 3.9, 15.5 mg/g) in healthy and diseased leaves respectively was recorded and in susceptible cv. Byadgi dabbi (20.1, 4.7, 24.8 mg/g and 9.53, 4.13, 13.66 mg/g) in healthy and diseased leaves was recorded respectively.

Sankar and Sreeramulu (2009) worked on biochemical alterations in teak plants exposed to biotic stress of powdery mildew infection caused by *Uncinula tectonae,* Significant gradual loss of chlorophyll content *viz.,* chlorophyll-a (1.86 and 1.25 mg/g), chlorophyll-b (2.87, 2.27 mg/g) and total chlorophyll (3.93, 3.29 mg/g) in healthy and diseased leaves respectively was recorded 30 days after inoculation. Similar results were observed on 10 and 20 days after inoculation.

Dhingra *et al.* (2013) experimented on cauliflower genotypes for biochemical parameters where moderately resistant genotype Pusa Sharda had higher total chlorophyll (2.44, 1.84 mg/g) while lowest in susceptible genotype 24-2 (2.18, 1.32 mg/g) in healthy and infected leaves respectively against *alternaria* blight.

Reddy and Sireesha (2013) experimented on enzyme activity and biochemical constituents of healthy and stem rot infected tissues of susceptible variety of groundnut TMV2. Results revealed that on 11^{th} day after inoculation total chlorophyll (1.496, 0.840 mg/g), chlorophyll-a (1.430, 0.616 mg/g) and chlorophyll-b (0.353, 0.226 mg/g) was observed in healthy plants and stem rot infected plants respectively.

Chavan and Suryavanshi (2014) analysed biochemical parameters in genotype JS-335 (susceptible) and MAUS-71 (resistant) in response to infection by pathogen *Colletotrichum truncatum* in soyabean. Chlorophyll-a, chlorophyll-b and total chlorophyll contents were higher in healthy leaves of resistant genotype MAUS-71 (0.8, 0.7, 1.7 mg/g and 0.8, 0.5, 1.5 mg/g) respectively in healthy and diseased leaves while in susceptible genotype JS-335 (0.7, 0.4, 1.3 mg/g and 0.3, 0.1, 0.5 mg/g) respectively in healthy and diseased plants.

Shulka *et al.* (2014) analysed the chlorophyll content in susceptible (NDM-1), one moderately resistant (Pusa Vishal) and resistant (UPM-98) genotypes of mungbean against anthracnose and revealed that the resistant and moderately resistant genotypes showed higher amounts of chlorophyll than the susceptible genotypes in both healthy and infected leaves. Per cent reduction of chlorophyll-a, chlorophyll-b, and total chlorophyll over healthy was recorded in case of UPM-98 (8.15, 12.09, 9.68 mg/g), Pusa Vishal (12.73, 27.64, 17.96 mg/g), NDM-1 (18.76, 37.4, 25.06 gm/g).

Mishra *et al.* (2015) conducted experiment on chlorophyll content index of wheat against yellow rust and reported per cent reduction of chlorophyll content of susceptible over resistant lines are 36.02 at early, 35.90 at medium early, 36.95 at medium late and 39.62 at later stages.

2.1.2 Phenols

It has been widely recognized that the aromatic compounds such as mono and dihydroxyphenols, phenolic glucoside, flavonoids, anthocyanin, aromatic amino acids and coumarin derivatives were increased in host tissues invaded by pathogen. One of the major biological properties of phenolic compounds is their antimicrobial activity and their main role in plants is to act as protective compounds against disease causing agents such as fungi, bacteria and viruses.

Phenolic compounds are common constituents of many plants. They include simple phenols, coumarin, mostly flavonoids and certain amino acids, prosthetic groups of some enzymes, plant pigments and complex derivatives such as lignins. Phenolic substances are known to participate in a number of biochemical processes, such as oxidation–reduction reactions and stimulation as well as, inhibition of auxin activity. Phenolic compounds occur in a variety of simple and complex forms. Simple phenols such as cinnamic acid, coumarin, caffeic, protocatechuic, chlorogenic and quinic acid exhibit antimicrobial activities. In many instances, there is a positive correlation between the amount of phenolic content and degree of resistance to plant disease.

Mitter *et al.* (1997) analysed phenol content in resistant genotype ICC-1096 and susceptible BGM-408 and they observed slightly more phenol content in healthy leaves than infected by *Botrytis cinerea* wherein, ICC-1069 recorded (3.6, 3.0 mg/g) and BGM-408 (2.2, 1.4 mg/g) in healthy and diseased leaves respectively.

Sankar and Jindal (2001) assed total phenol in grapes in response to infection by anthracnose caused by *Sphaceloma ampelinum*. Resistant variety H-144 recorded higher amount of total phenol (4.78, 5.64 mg/g) then moderately resistant variety Beauty Seedless (4.18, 4.64 mg/g) and susceptible variety Pusa Seedless (3.04, 3.15 mg/g) in healthy and diseased leaves respectively.

Jyosthna *et al.* (2004) worked on thirteen groundnut cultivars for biochemical parameters namely chlorophyll, total phenols, peroxidase and polyphenol oxidase were estimated. Higher amount of phenol content in infected leaves of resistant cv. FDRS-10 (4.7, 6.3 mg/100g), moderately resistant cv. K-134 (4.1, 5.5 mg/100g) and susceptible cv. TMV-2 (3.6, 4.4 mg/100g) were recorded in healthy and diseased leaves respectively. Increase in phenol content from 5 to 20th days after inoculation was recorded.

Ashtaputre (2005) studied biochemical parameters of healthy and diseased leaves of susceptible (Byadgi dabbi) and moderately resistant (Taiwan-8) varieties infected by powdery mildew caused by *Leveillula taurica* on chilli. Taiwan-8 showed higher phenol content (2.87, 1.5 mg/g) and there was no much difference in susceptible cv. Byadgi dabbi which recorded (2.65, 1.3 mg/g) in healthy and infected leaves respectively.

Dakshayani *et al.* (2005) screened six mungbean genotypes to know the biochemical changes in response to infection by powdery mildew and observed that TM-98-50 showed gradual increase in phenols from 30 (0.516 mg/g) to 50 (0.656 mg/g) days after sowing and later there was decrease in phenols till harvest (0.177 mg/g).

Sankar and Sreeramulu (2009) worked on biochemical alterations in teak plants exposed to biotic stress of powdery mildew infection caused by *Uncinula tectonae,* total phenol content was considerably increased in the infected leaves at 30 days after inoculation recorded (4.96 mg/g) than healthy ones (4.10 mg/g). Similar results were observed on 10 and 20 days after inoculation.

Aly *et al.* (2012) examined correlations between some biochemical components and powdery mildews (PMs) resistance in flax cultivars. Among nine cultivars, resistant cultivars had higher concentration of phenol (125.30 mg/g) compared to susceptible cultivars (54.66 mg/g).

Dhingra *et al.* (2013) experimented on cauliflower genotypes for biochemical parameters where moderately resistant genotype Pusa Sharda had higher phenol (14.91, 13.27 mg/g) whereas susceptible genotype 24-2 had (8.17, 6.30 mg/g) in healthy and infected leaves respectively against *Alternaria* blight.

Chavan and Suryavanshi (2014) worked on two genotypes JS-335 (susceptible) and MAUS-71 (resistant) after infection by pathogen *Colletotrichum truncatum* in soyabean. MAUS-71 (resistant) had total phenol (0.9, 1.1 mg/ g) and orthodihydroxy (OD) phenol (0.7, 0.9 mg/g) and JS-335 (susceptible) had total phenol (0.5, 0.6 mg/ g) and orthodihydroxy (OD) phenol (0.3, 0.4 mg/g) in healthy and resistant genotypes respectively.

Shukla *et al.* (2014) studied two genotypes NDM-1(susceptible) and UPM-98 (resistant) against mungbean anthracnose. UPM-98 had total phenol (25.07) and orthodihydroxy (OD) phenol (25.84) and NDM-1 had phenol (13.4) OD phenol (21.98) per cent increase of phenol over healthy.

Gurjar *et al.* (2015) evaluated thirty two grape genotype (12 parents and 20 hybrids) for natural incidence of powdery mildew for estimation of different biochemical parameters and reported that the resistant genotypes had higher phenols in both healthy and diseased leaves. Extremely resistance genotype *Vitis parviflora* had (4.38, 5.42 mg/g) followed by highly resistant genotype Male Hybrid (4.24, 5.15 mg/g) compared to extremely susceptible genotype Hybrid Seedless, which had the least phenol content (2.87, 3.47 mg/g) in healthy and diseased leaves respectively.

2.1.3 Sugars

Mitter *et al*. (1997) conducted experiment on healthy plants of chickpea genotype ICC 1069, resistant to *Botrytis cinerea* and recorded lower soluble sugar (11.6, 5.0 mg/g) than susceptible BGM-408 (19.3, 14.3 mg/g) in healthy and diseased leaves respectively.

Dakshayani *et al.* (2005) screened six genotypes to know the biochemical changes in response to infection by powdery mildew and observed higher levels of reducing, non-reducing and total sugars in the susceptible genotype cv. Chinamung (8.24, 1.69, 9.93 mg/g), Pusa baisakhi (9.65, 2.31, 11.96 mg/g) and TM-98-50 (10.23, 3.07, 13.30 mg/g) compared to the resistant genotype TARM-18 (4.12, 2.58, 6.69 mg/g) at 30 DAS in healthy and diseased plants respectively.

Dhanumjayarao *et al.* (2006) worked on biochemical variability studies for disease resistance in grape against powdery mildew. Resistant pearl of csaba showed reducing sugar content (2.54, 2.14 mg/g) total sugar (3.45, 3.32 mg/g), while susceptible tas-a-ganesh which recorded reducing sugar (3.00, 2.09 mg/g) total sugar (4.80, 3.32 mg/g) and highly susceptible perlette showed reducing sugar (3.27, 2.05 mg/g) total sugar (4.63, 3.79 mg/g)in healthy and diseased leaves respectively.

Sunkad and Kulkarni (2006) studied the mechanism of resistance on the basis of structural and biochemical changes in resistant (GPBD- 4 and DH-22), moderately resistant (K-134 and R-8808) and susceptible (KRG-1 and TMV-2) genotypes of groundnut to *Puccinia arachidis* it was found that DH-22 showed lower content of reducing sugar (11.32, 13.76 mg/g), non-reducing sugars (2.39, 2.49mg/g) and total sugars (13.71, 16.25 mg/g) while TMV-2 showed higher content of reducing sugar (9.69, 11.62 mg/g), non-reducing sugars (1.60, 1.66 mg/g) and total sugars (12.43, 13.28 mg/g) in healthy and diseased leaves respectively.

Mahatma *et al*. (2009) conducted biochemical experiment on pearl millet genotypes, which was carried at pre (45 DAS) and post infection (57 DAS i.e. 7 days after infection) stage. Total soluble sugar was greater at pre infection than post infection in downy mildew resistant and susceptible genotypes of pearl millet. In the resistant cultivar J-2290 recorded higher amount of total soluble sugar (8.16, 3.03 mg/g) at pre and post infection stage respectively. Susceptible cultivar J-2296 had comparatively lesser amount of total sugar (8.93, 8.50 mg/g) at pre and post infection stage respectively.

Sankar and Sreeramulu (2009) worked on biochemical alterations in teak plants exposed to biotic stress of powdery mildew infection caused by *Uncinula tectonae,* where after 30 days of inoculation reducing sugar (7.84, 6.16 mg/g) and non-reducing sugar (3.90, 2.84 mg/g) in healthy and infected plants respectively. Similar results were obtained on 10 and 20 days after inoculation.

Patil *et al.,* (2011) conducted biochemical experiment on wheat resistant and susceptible genotypes DWR (resistant) showed higher sugar (14.19 mg/g) whereas, DDK 1025 (susceptible) showed less (11.48 mg/g) against spot blotch disease.

Dhingra *et al.* (2013) experimented on cauliflower genotypes for biochemical parameters where moderately resistant genotype Pusa Sharda had higher total sugar (62.46, 56.22 mg/g) and susceptible genotype 24-2 (48.26, 36.44 mg/g) in healthy and infected leaves respectively against A*lternaria* blight.

Shulka *et al.* (2014) analysis of sugars in susceptible (NDM-1), one moderately resistant (Pusa Vishal) and resistant (UPM-98) genotypes of mungbean against anthracnose and revealed that, UPM-98 (7.19, 10.27, 8.17 mg/g), Pusa Vishal (5.69, 12.89, 8.52 mg/g) and NDM-1 (9.96, 21.95, 14.7 mg/g) percent reduction of reducing, non-reducing and total sugar respectively over healthy.

Chavan and Suryavanshi (2014) worked on two genotypes JS-335 (susceptible) and MAUS-71 (resistant) after infection by pathogen *Colletotrichum truncatum* in soyabean. Total sugar, non reducing and reducing sugar contents were higher in MAUS-71 (5.3, 1.6, 3.7 mg/g and 4.9, 1.5, 3.4 mg/g) and JS-335 (7.0, 2.4, 4.6 mg/g and 6.4, 2.2, 4.2 mg/g) in healthy and infected leaves respectively.

2.2 Changes in the proteins and amino acids.

2.2.1 Protein

Sunkad and Kulkarni (2006) studied the mechanism of resistance on the basis of structural and biochemical changes in resistant (GPBD- 4 and DH-22), moderately resistant (K-134 and R-8808) and susceptible (KRG-1 and TMV-2) genotypes of groundnut to *Puccinia arachidis* was studied. DH-22 (6.47, 4.79 mg/g), KRG-1 (3.21, 3.54 mg/g) and TMV-2 (3.92, 3.90 mg/g) in healthy and diseased leaves respectively.

Sankar and Sreeramulu (2009) worked on biochemical alterations in teak plants exposed to biotic stress of powdery mildew infection caused by *Uncinula tectonae,* protein content found to be higher in healthy leaves, at 30 days after inoculation recorded (64.3, 38.3 mg/g) in healthy and diseased leaves respectively. Similar results were obtained on 10 and 20 days after inoculation.

Ashry and Mohamed (2011) worked on various physiological defenses including secondary metabolites, proline, total soluble protein and antioxidant enzymes were investigated in leaves and stems of eighteen flax lines either resistant or susceptible to powdery mildew. Higher concentration of soluble protein in resistant parent observed was in the range of 39.11 to 50.08 mg/ml and comparatively less concentration of total soluble protein was observed in the parents ranging from 21.10 to 36.78 mg/ml.

Parashar and Lodha (2011) quantified the protein in fennel (*Foeniculum vulgare*) infected with *Ramularia* blight and powdery mildew. The content of proteins was recorded in healthy and diseased leaves. Different plant parts showed variation in their protein contents. In diseased leaves relatively higher (0.860) concentration of protein was observed than the normal leaves (0.774).

Aly *et al.* (2012) examined correlations between some biochemical components and powdery mildews (PMs) had resistance in flax cultivars. Among nine cultivars, resistant cultivar ottowa-770B had higher concentration of protein (62.22 mg/g) compared to susceptible cultivar C.I.2008 (44.07 mg/g).

Dhingra *et al.* (2013) analysed cauliflower genotypes for biochemical parameters wherein, moderately resistant genotype Pusa Sharda had higher protein (17.30, 15.47 mg/g) and susceptible genotype 24-2 (11.42, 8.43 mg/g) in healthy and infected leaves respectively against A*lternaria* blight.

Shukla *et al.* (2014) analysed protein content in mung bean genotype *viz.,* NDM-1(susceptible) and UPM-98 (resistant) against anthracnose. UPM-98 recorded less protein content 13.2 mg/g in healthy leaves compared to diseased leaves 15.7 mg/g. Simillar kind of results observed in susceptible genotype but the concentration was comparatively less.

2.2.2 Amino acids

Mitter *et al.* (1997) analysed amino acid content in resistant genotype cv. ICC-1096 and susceptible cv. BGM-408 and they observed less of amino acid content in infected leaves than healthy leaves. Resistant genotype cv. ICC-1069 recorded less amino acid (219.6, 270 mg/g) compared to BGM-408 (310.3, 1179.3 mg/g) in healthy and diseased leaves.

Mahatma *et al*. (2009) characterized pearl millet genotypes for biochemical composition against downy mildew infection. They observed 2-2.5 per cent higher amino acid content in susceptible genotypes whereas, resistant genotypes possessed 6.2-76 per cent higher amino acid than their constitutive level.

2.3 Quantitative status of nutrients due to pathogenicity.

2.3.1 Minerals

The mineral nutrition is one of the basic processes that may be impaired by disease. The minerals are generally essential for the growth of the plants. These minerals function as cellular components, activator, inhibitor and regulator of metabolism.

The alteration in mineral composition due to infection by plant pathogen has been reported by many workers.

Hegde and Munjal (1971) reported a significant change in elemental composition of French bean pod infected with *colletotrichum lindemuthianum.* They reported significant reduction in N(3.43, 3.29), P(0.53, 0.47), K(1.58, 1.37), Ca(0.30, 0.28), Mg(0.28, 0.25), Cu(19, 16), Zn(107, 94) and Mn(36, 33) content, while a significant increase in Fe(103, 116) content in diseased plants when compared to healthy plants.

Wheeler (1975) reported loss of carbohydrate, nitrogenous minerals P and K from oat leaves pretreated with victorin, a toxin produced by *Helminthosporium victoriae.*

Philip and Devadath (1980) working with physiology of bacterial blight infected tolerant and susceptible rice cultivars, found that total nitrogen, P, K and Ca was much higher in healthy plants when compered to infected ones. But Fe and Mg content was more in infected leaves than healthy leaves of all cultivars.

Sindhan and Parashar (1981) analysed 12 pea cultivars showing variable degree of powdery mildew incidence, and found a high significant negative correlation between P, K, Mg, Zn, Cu and disease intensity, whereas a highly significant positive correlation was observed between N, Ca, Mn, Fe and disease intensity. The resistant leaves had higher concentration of P, K, Mg and Cu and fewer amounts of N, Ca, Mn and Fe.

Thite *et al.* (2013) studied on various inorganic constituents in *Dalbergia sissoo* powdery mildew and reported N (1.73, 1.17), P (0.96, 0.64), K (1, 1.35), Ca (0.9, 1.2), Mg (0.44, 0.28), S (0.1, 0.09), Na (0.35, 0.5), Zn (1.06, 1.30), Fe (12.94, 9.29), Cu (0.23, 0.35), Mn (4.02, 2.61), Mo (0.0096, 0.0052) and B (0.58, 0.62) Per cent in healthy and infected leaves.

2.4 *In vivo* evaluation of fungicides

2.4.1 Fungicide

Suresh and Padaganur (1990) conducted an experiment with six fungicides among them tridemorph (0.02 %) resulted in a reduction of the disease by 87.72 per cent against powdery mildew of greengram.

Dhruj *et al*. (1996) evaluated eight fungicides *viz.,* propiconazole, penconazole, hexaconazole, tridemorph, triadimefon, dinocap, sulphur and sulphur dust for the control powdery mildew of fenugreek and reported that minimum disease intensity (19.38%) and highest yield (2132 kg/ha) were recorded by penconazole @ 0.01 % followed by hexaconazole @ 0.005 % and propiconazole @ 0.025 % against powdery mildew of fenugreek (*Trigonella foenum-graecum*) caused by *Leveillula taurica* and *Erysiphe polygoni.*

Gupta and Shyam (1998) evaluated efficacy of six new fungicides, viz., triadimefon, hexaconazole, difenconazole, flusilazole, fenarimol and penconazole along with mancozeb and chlorothalonil was tested for the control of pea powdery mildew and rust. Three sprays were applied at fortnightly intervals recorded triadimefon (0.05 %) found highly effective in reducing the severity (0.00 PDI) of powdery mildew while hexaconazole (0.10 %) difenconazole (0.015 %) and flusilazole (0.04 %) were best against powdery mildew besides exhibiting an appreciable increase in green pod yield.

Audichya and Thakare (2000) determined the efficacy of 4 systemic fungicides (carbendazim, triadimefon, iprobenfos and PI-IMA) for the contrast of powdery mildew of opium poppy caused by *Erysiphe polygony*. Carbendazim (0.05, 0.1 %) as protectant and curative found to reduce diseased incidence upto 70.79 % and 78.34 % respectively and obtained higher seed yield 20.67 %, and 25.10 % respectively over control.

Singh and Pal (2000) experimented on wheat powdery mildew and reported three sprays of Bayleton 25% WP (0.05 %) at stem elongation, flag leaf and flowering stages provided significantly effective control of the disease to the extent of 69.54 per cent followed by Tilt with 67.82 per cent.

Saxena and Saxena (2002) reported that some of the tested fungicides like penconazole (0.05 %), carbendazim (0.05 %) and tridemorph (0.075 %) were effectively controlled powdery mildew (*E. polygoni*) of mung bean (*Vigna radiata*) and best recorded from carbendazim with disease reduction by 74.5 % and highest yield of 480 kg/ha .

Sharmila *et al*. (2004) tested few fungicides against the powdery mildew (*Leveillula taurica*) of chilli cv. Byadgi Kaddi with carbendazim, difenconazole, hexaconazole, penconazole, propiconazole, triadimefon and wettable sulfur at 0.1 % and found all the tested fungicides found to control the disease incidence with increased yield compared to untreated control. The best results were obtained with the spraying of penconazole (9.03 %), followed by propiconazole (13.84 %) with the highest yields of 7.97 and 7.40 q/ha respectively. However, carbendazim (1:8.44) and wettable sulfur (1:8.43) were also considered to be the most economical fungicides.

Upasana *et al*. (2005) conducted a study with the systemic fungitoxicants for the control of powdery mildew (*Erysiphe graminis* f.sp. *tritici*) of wheat cv. PBW 343. But reported with significantly less disease index during both the years compared to unsprayed plots. Although, per cent disease control ranged from 37.2 to 100 per cent over the control the disease was completely controlled with foliar application of triadimefon and propiconazole at 0.050 and 0.075 per cent respectively. In all the treatments increase in yield was observed during both years. Propiconazole and triadimefon at all concentrations recorded the highest mean yields (57.25-60.45 and 57.21-59.55 q/ha respectively).

Shivanna *et al.* (2006) tested the efficacy of eight systemic, two non-systemic fungicides and one botanical under field condition for the control of powdery mildew of okra and penconazole at 0.1 per cent was found to be the best fungicide which recorded least disease incidence (3.7 %) followed by hexaconazole (5.8 %) and propiconazole (6.8 %).

Ashtaputre *et al.* (2007) tested the efficacy of triazoles and were found most effective in reducing powdery mildew of chilli (*Leveillula taurica*). Among them penconazole (9.27 PDI) reduced the disease effectively and enhanced the yield (12.72 q/ha) followed by triadimefon, propiconazole, hexaconazole and difenconazole.

Suryawanshi *et al.* (2009) evaluated fungicides against powdery mildew of mung bean and reported that Karathane 48 EC (@ 0.1 %) was most effective and recorded least mean powdery mildew intensity (15.14 %), highest grain yield (1425 kg/ha) and test weight (58.00 g).

Adinarayan *et al*. (2012) tested the efficacy of Penconazole 10 EC against powdery mildew in Urdbean, results showed that penconazole @ 1.0 mL/L was found almost free from powdery mildew with the lowest PDI of 1.60 and it was significantly superior over the rest of the concentrations. The next best treatment was Penconazole 10 EC (NS) @ 0.6 mL/L (PDI – 7.46) which was significantly superior over rest of the treatments.

Akhileshwari *et al.* (2012) tested the efficacy of eight fungicides against sunflower powdery mildew caused by *Erysiphe cichoracearum.* Among them Difenconazole 25 EC @ 0.005 % recorded 78.29 % and 88.64 % disease control after I and II spray respectively, with higher yield 12.24 q/ha.

Ramesh *et al.* (2013) screened six fungicides against *Leveillula taurica* causing powdery mildew in chilli. Results revealed that mean Per cent disease index (13.03 %) and higher yield (12.72 q/ha) was recorded by Penconazole @ 0.1 % concentration.

Ajithkumar *et al.* (2014) evaluated the combi-fungicide UPF-509 (Azoxystrobin 8.3% + Mancozeb 66.7 %) 75%WG *In vivo* to know the efficacy at 1200, 1500 and 1800 g/ha along with recommended fungicides against powdery mildew and anthracnose diseases in chilli during *kharif* seasons of 2011 and 2012. Among the eight treatments, UPF-509 at 1800 g/ha proved to be best for the management of powdery mildew (11.33 PDI) and anthracnose (6.67 PDI), which was superior over all other treatments with maximum fruit yield of 21.91 q/ha.

Devi and Prakasam (2014) conducted field trials to determine the bioeffficacy of Azoxystrobin 25 SC against powdery mildew of chilli. Three sprays with Azoxystrobin at 150 a.i, 125 g a.i and 100 g a.i/ha were tried on chilli for controlling the spread of *Leveillula taurica*. The results for the first and second season revealed that maximum control of powdery mildew disease was by Azoxystrobin at 150 g a.i/ha gave the maximum disease reduction of about 91.10 and 86.07 on leaves respectively.

Ahir *et al.* (2015) evaluated with nine fungicides against powdery mildew of green gram and reported that minimum disease intensity (12.94 %) and highest yield (923.93 kg/ha) with the application of hexaconazole @ 0.05 % which was statistically at par with penconazole @ 0.05 % which gave 16.81 per cent disease intensity and 846.23 kg/ ha seed yield.

Channaveeresh and Kulkarni (2015) screened twelve fungicides against *Erysiphe polygoni* causing powdery mildew in black gram, among them azoxystrobin 250 % SC at 0.1 per cent concentration (94.16 %) inhibited maximum conidial germination and it was followed by hexaconazole 5 % EC (90.51 %).

Sushmita (2015) screened fungicides *in vitro* against the *Leveillula taurica* causing powdery mildew in chickpea, revealed that among systemic fungicides myclobutanil and azoxystrobin showed complete inhibition of conidial germination at 0.05, 0.1 and 0.15 per cent concentration. In combi product (Captan 70 % + Hexaconazole 5 %) 75WP at 0.15 per cent concentration reduced maximum inhibition of spore germination (89.83 %).

3. MATERIAL AND METHODS

The present investigation on chickpea powdery mildew was carried out during *rabi* 2015-16. The results of the experiment conducted on various aspects of chickpea powdery mildew caused by *Leveillula taurica* (Lev.) Arnaud with reference to analysis of chlorophyll, phenols, sugars, proteins, amino acids and nutrient status of chickpea. *In vivo* evaluations of newer molecules of fungicides were conducted at Agricultural Research Station (ARS), Annigeri during *rabi* 2015-16. For biochemical and nutrient analysis healthy leaves and diseased leaves were collected after 63, 70, 77, 84 and 91 days after sowing (DAS) from Regional Agricultural Research Station (RARS) Vijayapur .

3.1 Studies on biochemical parameters in chickpea

Biochemical analysis of chlorophyll, phenol, total sugars, reducing sugars, proteins and amino acids were analyzed in healthy leaves against the powdery mildew infected leaves of chickpea are mentioned here under.

3.1.1 Extraction of leaf material in alcohol

Leaf material was extracted in ethanol as per the procedure followed by Jaypal and Mahadevan (1968). Estimation of metabolites requires their complete extraction from the tissues. The activities of the enzymes, which synthesize and utilize them, need to be stopped at once to get reliable values. Plant constituents possess different degrees of solubility in different solvents. Though water is the universal solvent, it does not penetrate tissues quickly to stop enzymatic activity. In this context, alcohol, especially warm alcohol is the choice solvent for the extraction.

Reagent:

Distilled ethanol (80 %)

Procedure:

About 5 g of chickpea leaf was weighed and cut into small pieces and plunged immediately into boiling alcohol. The tissue was extracted in a boiling water bath, cooled and the extract was passed through double layer of muslin cloth. The pieces of the tissue were homogenized thoroughly using a mortar and pestle with a little warm alcohol. Homogenate was filtered through the muslin cloth and this step was repeated once more. The filtrates were pooled and filtered through Whatman filter paper No.1 and volume was adjusted to 10 ml with 80 % alcohol. The filtrates were stored at 4˚C. This alcoholic extract was used further for estimation of total sugar, reducing sugar, non - reducing sugar, total phenol, amino acid and soluble protein.

3.1.2 Estimation of chlorophyll content

One hundred milligrams of healthy and infected leaf samples of chickpea were collected from the field. The chlorophyll was extracted in Dimethyl sulfoxide (DMSO) as described by Hiscox and Israelstan (1979). The leaf samples were placed in test tube containing 7 ml DMSO and incubated at room temperature for 24 hr. The extracted liquid volume was adjusted to 10 ml with DMSO, further

the stock solution was diluted to 50 per cent with DMSO. About 3.0 ml of the sample of chlorophyll extract was transferred to cuvet and OD values at 645 and 663 nm were recorded against DMSO blank with the help of Spectrophotometer. Chlorophyll content was calculated by following the equation given by Hiscox and Israelstan (1979).

$$\text{Total Chlorophyll} = 20.2\,(A\,645) + 8.02\,(A\,663) \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\ \text{fr.wt.})$$

$$\text{Chlorophyll (a)} = 12.7\,(A\,663) - 2.69\,(A\,645) \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\ \text{fr.wt.})$$

$$\text{Chlorophyll (b)} = 22.9\,(A\,645) - 4.68\,(A\,663) \times \frac{V}{1000 \times W \times a}\ (\text{mg g}^{-1}\ \text{fr.wt.})$$

Where,

V = Volume of the extract (10 ml)

W = Fresh weight of the sample (200 mg)

a = Path length of light (1 cm)

A_{645} = Absorbance of the extract at 645 nm

A_{663} =Absorbance of the extract at 663 nm

3.1.3 Estimation of reducing sugars

Reducing sugar content from leaf samples were estimated by Nelson's modifications of Somogyi's method (Nelson, 1944).

Reagents:

1. Alkaline Copper Reagent

Solution A: 25 g of anhydrous sodium carbonate, 25 g of sodium potassium tartrate, 20 g of sodium bicarbonate and 20 g of sodium sulfate were dissolved separately in distilled water and diluted to 1 liter.

Solution B: 15 g of copper sulfate was dissolved in distilled water, one or two drops of concentrated sulphuric acid was added and the volume was made upto 100 ml with distilled water.

Solutions A and B were mixed in 24:1 (v/v) proportion just before use.

2. Arsenomolybdate Reagent

Twenty five gram of ammonium molybdate was dissolved in 450 ml of distilled water and 21 ml of concentrated sulphuric acid. Three grams of disodium hydrogen arsenate was dissolved in 25 ml of distilled water. The two solutions were mixed with stirring and placed in an incubator at 37 ˚C for 24-48 hrs. The reagent was stored in amber colored bottle as it remains stable for a few months in such bottles.

3. Standard sugar solution

100 mg of D-glucose was dissolved in a little amount of distilled water and volume made up to 100ml in a volumetric flask. This solution contained 1 mg of glucose per ml.

Procedure:

1. In a series of labelled test tubes, suitable aliquots of working standard (10-100 µg) were pipetted out (for preparation of standard graph) and 0.2 ml of each leaf extract was taken from 10 ml of alcoholic extract and made up to 1 ml with distilled water in all the test tubes. A reagent blank was maintained with 1 ml of distilled water.
2. One milliliter of freshly prepared alkaline copper reagent was added to all the tubes including reagent blank, mixed well and placed in boiling water bath for exactly 20 minutes. The tubes were cooled under tap water without shaking and 1 ml of arsenomolybdate reagent was added to all the tubes and mixed immediately till the effervescence dried.
3. The volume was made up to 10 ml with distilled water and density of the blue colored complex was read at 510 nm against reagent blank.
4. The amount of reducing sugar present was calculated from the standard graph of glucose and the values were expressed as mg of reducing sugar present in per gram of sample.

3.1.4 Estimation of total sugars

Non reducing sugar was hydrolyzed using 1 ml of 1N H_2SO_4 and then estimated as in case of reducing sugars to get the total sugars.

Reagents:

1. 0.1N, 1.0N HCL and 1 N NaOH
2. Phenolphthalein indicator solution in alcohol.

Procedure:

1. One ml of each leaf extract was taken in series of test tubes from 10 ml of alcoholic extract and 1 ml of 1 N HCl was added and placed in a water bath maintained at 50-60˚C for exactly 20 min. The tube was cooled a drop of phenolphthalein indicator solution was added and mixed well.
2. NaOH 1 N was added drop wise till the solution turned to pink.
3. HCl 0.1 N was added drop wise till the solution became colorless.

4. The contents of the tube were made up to 10 ml with distilled water.
5. 0.2 ml of this aliquot was used to estimate reducing sugar present in the hydrolysate by Nelson – Somogyi's method.
6. The quantity of reducing sugar was subtracted from that of total sugar and multiplied by a conversion factor of 0.95 to get non-reducing sugar.

3.1.5 Estimation of amino acids

Total free amino acids in ethanol extracts were estimated by nin-hydrin method (Moore and Stein, 1958).

Materials:

a) Nin-hydrin: 0.8g stannous chloride ($SnCl_2.2H_2O$) was dissolved in 500ml of 0.2 M citrate buffer (pH 5.0). This solution was added to 20g of nin-hydrin in 500 ml of methyl cellosolve (2-methoxyethanol).

b) 0.2 M citrate Buffer pH 5.0.

c) Diluent solvent: Equal volumes of water and n-propanol were mixed and used

Procedure:

1. 0.2 ml of each leaf extract was taken and 1ml of nin-hydrin solution was added to each test tube and volume was made 2 ml with distilled water.
2. The reagent blank was prepared with 2ml distilled water.
3. Tubes were heated in a boiling water bath for 20 min.
4. The 5ml of the diluent solution was added and mixed.
5. After 15 min, read the intensity of purple colour against a reagent blank in a colorimeter at 570 nm.

Standard solution:

50 mg leucine was dissolved in 50 ml of distilled water in a volumetric flask. Ten ml of this stock standard was taken and diluted to10 ml in another volumetric flask for preparation of working standard solution. A series of volumes from 0.1 to 1 ml of this standard solution gives concentration range from 10 µg to 100 µg.

3.1.6 Estimation of soluble protein:

Total protein in ethanol extracts of leaves from healthy and powdery mildew infected were estimated by the procedure given by Lowry *et al*. (1951).

Materials:

1. Alkaline copper reagent

   Solution A-2 % sodium carbonate in 0.1 N NaOH
   Solution B-1 % sodium potassium tartrate
   Solution C-0.5 % copper sulfate
   Solution A, B and C were mixed in 100:1:1 proportion just before use.

2. Stock protein standard solution

50 mg Bovine Serum Albumin (BSA) was dissolved in distilled water and made up to 50 ml with distilled water in a volumetric flask. This solution contained 1 mg of protein per ml.

3. Working standard solution

Ten ml of stock standard solution was diluted to 100 ml with distilled water in a volumetric flask. This solution contained 100 µg of protein per ml.

4. Folin-Ciocalteau reagent (1N)

Commercially available FCR (2N) was diluted suitably to get 1N FCR with distilled water.

Procedure:

1) Into a series of labeled test tubes, working standard solution (20-100 mg) of protein was pipetted out. Distilled water was added to make up to 1 ml each. One blank tube with 1 ml of distilled water was maintained.
2) 5 ml of alkaline copper reagent was added to all tubes, mixed thoroughly and allowed to react for 10 min.
3) 0.5 ml of 1N FCR was added to all the tubes, mixed and kept in dark for 30 min.
4) Suitable aliquots of the unknown sample were treated as explained above.
5) The per cent T values of the standard and the sample were recorded against reagent blank and was set to 100 % T at 660 nm.
6) The mg of protein per g of sample was calculated from the standard graph using Bovine serum albumin (BSA) fraction V.

3.1.7 Estimation of total free phenol

Total free phenols in the clarified ethanol extract of leaves from healthy and powdery mildew infected were estimated by Folin Ciocalteau Reagent method (Bray and Thorpe, 1954)

Reagents:

1. Sodium Carbonate Solution

Two grams of sodium carbonate was dissolved in 0.1 N NaOH and made up to 100 ml with 0.1 N NaOH.

2. Folin-Ciocalteau Reagent (1 N)

3. Standard Catechol Solution

15 mg of catechol was dissolved in distilled water and made up to 50 ml with distilled water in a volumetric flask. This solution contained 50 µg of catechol per ml.

Procedure:

1. In a series of labeled test tubes, 10-50 µg of working standard solution was pipetted out (for preparation of standard graph) and 0.2 ml of each alcoholic leaf extract was taken in a test tube and the volume was made up to 1 ml with distilled water in all the test tubes. A blank with 1 ml distilled water was maintained.

2. One ml of 1N FCR was added to all the tubes, mixed well and 2 ml of sodium carbonate solution was added to all the test tubes and mixed well.

3. The tubes were placed in a boiling water bath for exactly 1 min, cooled and made up to 15 ml with distilled water.

4. The per cent T of the standard and sample was read against reagent blank which was adjusted to 100 % T at 650 nm.

5. The total free phenol content of the sample was calculated from the standard graph and expressed as mg per gram.

3.2 Studies on nutrient parameters of chickpea

Nutrient analysis of N, P, K, Mg, Zn, Fe, Mn, Cu, and B were analysed in healthy leaves against the powdery mildew infected leaves of chickpea was analysed at department of soil science and agricultural chemistry, UAS Dharwad are mentioned here under.

3.2.1 Total nitrogen (%)

Total nitrogen was estimated by Kjeldal method (Jackson, 1973), 0.5 g of dried sample was weighed and transferred to 100 ml kjeldal flask, 10 ml of conc. H_2SO_4 and 2 to 3 g of digestion mixture or catalytic mixture was added. The contents were digested in a fume cupboard over a low flame and then over strong flame till light bluish green colour was obtained. Then the contents were cooled and the volume was made upto 50 ml with distilled water. 10 ml of digested sample was pipetted into a microkjeldhal to which 10 ml distilled water was added. 20-25 ml of Boric acid solution was taken in a receiving flask and connected to receiving tube in such a way that the receiving tube was dipped in Boric acid. About 10 ml of 40 per cent NaOH was added to the distilled flasks and the contents were distilled. After complete distillation, the receiving flask was disconnected and content was titrated in receiving flask against standard acid till it became pink. Then the burette reading was taken and per cent 'N' was calculated using the formula.

$$N\ (\%) = \frac{BR \times N.\ of\ acid \times 0.014 \times Vol.\ of\ digested\ sample}{Weight\ of\ the\ sample \times aliquot\ taken} \times 100$$

Where,

Weight of the sample taken for digestion = 0.5 g

Volume of digested = 50 ml

Aliquot of plant digest taken for distillation = 10 ml

Normality of H_2SO_4 used in titration = 0.05 N

Constant value = 0.014

Burette reading = B.R.

The crude protein content was computed by using the formula.

Crude protein (%) = 'N' content of leaves x 6.25

3.2.2 Phosphorus

The concentration of P in the plant sample is usually about one tenth of the conc. of N. The medium level of P in most of the crops is less than 0.30 % on dry weight basis. Plant phosphorus is converted into orthophosphate during digestion. These orthophosphates react with molybdate and vanadate and give yellow coloured unreduced vando-molybdo-phosphoric heteropoly complex in nitric acid and medium. The yellow colour is attributed to substitution of oxyvanadium and oxymolybdium radicals for the oxygen of phosphate. The intensity of yellow colour is directly proportional to the concentration of phosphate present in the sample, which can be read in spectrophotometer. Yellow colour is developed in 30 min. and is stable for 2 to 8 weeks.

Calculation and observation

Weight of plant sample = W g

Volume of plant digest = V ml

Volume of digest taken for colour development = V_1 ml

Volume made up after colour development = V_2 ml

Conc. of P in aliquot as obtained from std. curve = ppm

$$\% P = \frac{\text{ppm from graph}}{10^6} \times \frac{\text{Volume of digest (V)}}{\text{Wt of plant sample (W)}} \times \frac{\text{Volume made after colour development}}{\text{Aliquot taken for colour development } (V_1)} \times 100$$

$$\% P = \frac{\text{ppm}}{10000}$$

3.2.3 Potassium

The most common method *i.e.*through flame photometry was used to estimate the potassium. When the aliquot of plant digest containing alkali and alkaline earth metallic cations is fed to the flame photometry, characteristic radiations are emitted. The characteristic colour and wavelength of radiation indicates the type of element. The intensity of emitted radiation indicates the concentration of that particular element in the sample and can be measured by photosensitive detector or photocell of the flame photometer.

$$\% K = \frac{\text{ppm from graph (I-II)}}{10^6} \times \frac{\text{Volume of digest (V)}}{\text{Weight of plant sample (W)}} \times \text{Dilution factor} \times 100$$

Where,

Weight of the plant sample = Wg

Volume of plant digest = Vml

Dilution factor

Conc. of K from std. curv (for sample) = ppm-I

Conc. of K for blank = ppm-II

3.2.4 Determination of Magnessium

Plant Mg from stable complex with versenate or EDTA at different pH Values. EDTA is a chelating agent and can be conveniently used for the determination of Mg. The influences of interfering ions of plants digest are avoided by using 2 % zirconium oxychloride solution. Plant digest is titrated with standard versenate solution using metal sensitive indicators in the presence of buffers. Titre values for Mg and it will indicate the content Mg in plant sample. Mg is determined by difference.

$$\% \text{ Mg in plant sample} = \frac{A}{W} \times \frac{C}{B} \times \text{Normality of EDTA} \times (F-H) - (G-I) \times \frac{\text{Eq. Wt. of Mg}}{D} \times \frac{100}{1000}$$

Where,

W – Wt. of the sample (g)

A – Volume made after digesting the plant sample (ml)

B – Aliquot of diacid or triacid digest taken for removing interfering ions (ml)

C – Volume made after removing interfering ions (ml)

D – Aliquot taken for titration against standard EDTA for Mg determination

F – Sample titre value for Mg estimation (ml)

G – Sample titre value for Caestimation (ml)

H – Blank titre value for Mg (ml)

Normality of standard EDTA = 0.01 N

3.2.5 Copper, Zinc, Ferrous and Manganese

Zn, Fe, Mn and Cu were estimated in plant digest either in diacid (HNO_3:$HClO_4$) or in triacid (HNO_3:H_2SO_4:$HClO_4$-10:1:4). The diacid or teiacid digest of plant material was fed directly to atomic absorption spectrophotometer with respective cathode lamps (Zn, Fe, Mn and Cu) with suitable dilutions if necessary and concentration of these elements was recorded in ppm by referring to standard curve.

$$\text{Total (Zn, Fe, Mn and Cu)} = \frac{\text{Volume made after digestion}}{\text{Wt of plant sample used for digestion}} \times \text{ppm from the instrument or graph}$$

$$\%\ \text{Zn, Fe, Mn and Cu in plant sample} = \frac{\text{ppm}}{10000}$$

3.2.6 Boron

The finely ground plant sample was wetted with saturated calcium hydroxide solution and then evaporated to dryness. It was an initial ignited on a burner and then placed in a muffle furnace at 550 c. The residue containing B was dissolved in measured volume of 0.1 N HCl. The suspension was centrifuged to obtain a clear test solution. B dissolved in HCl was reacted with curcumin to form a coloured complex for the colorometric determination of boron.

Procedure for Dry Ashing

Transfer 0.5 to 2.0 g of finely ground oven dry (at 70^0C) plant sample into silica or platinum dish. Wet the sample with 5 ml of saturated $Ca(OH)_2$ 2 % solution and evaporate the contents to dryness in an oven at 105^0 C. Remove the dish and ignite on a gas burner carefully. Then place the evaporating dish into a muffle furnace and ignite at 550^0 C for an hour to obtain a white or gray ash.

After dry ashing, remove the platinum or silica dish from muffle furnace and cool to room temperature. Add exactly 10 or 15 ml of 0.1 N HCl and stir with glass rod and also triturate with policeman to dissolve boron adhering to the sides of dish. Centrifuge the suspension for 10 minutes at 2400rpm to obtain a clear test solution. Similarly prepare a blank test solution without plant material.

Formation of Boron – Curcumin Complex:

1. Transfer 1.0 ml of plant test solution obtained after dry ashing into 50 ml beaker or conical flask.
2. Add 4 ml of curcumin-oxalic acid solution and mix the contents by swirling.
3. Evaporate the solution to dryness on a water bath regulated at 55^0 C. thereafter bake the residue for 15 minutes at 55^0 C to ensure complete dryness. During evaporation there is development of “rosecyanine” coloured substance.
4. Cool the residue and add 25 ml of 95% ethanol. Triturate the contents and filter through whatman filter paper directly into colorimeter yube.
5. Read the intensity of colour at 540 nm wavelength within 2 hours of the development of boron-curcumihn complex.
6. Fallow si8milar procedure for standards taking appropriate aliquot of B standard (50 ppm) to prepare 0-5 ppm B standards, and adding 4 ml of curcumin-oxalic acid solution evaporating to dryness and so on.

7. Carry out similar procedure for blank test solution also [$Ca(OH)_2$ digested solution without plant sample].
8. Draw a standard curve for boron by plotting absorbance values of standards boron curcumin coloured complex on Y axis and boron conc. on X axis.
9. Refer to standard curve and locate the boron conc. in plant test solution in ppm.

$$\text{B in plant sample (ppm)} = \frac{(A-B) \times C}{D \times W}$$

Where,

W- Weight of plant sample for dry ashing

A- ppm of boron from standard curve in sample test solution

B- ppm of boron from standard curve in blank solution

C- Volume of 0.1 N HCl used for dissolving in plant sample after dry ashing in muffle furnace

D- Aliquot of plant sample test solution taken for developing boron – curcumin complex

3.3 Field evaluation of fungicides

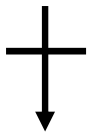
A field experiment was conducted during Rabi 2015-16 at Agricultural Research Station, Annigeri. The experiment was laid out in completely randomized block design (RCBD) with 10 treatments and replicated thrice. In each treatment five plants were tagged and the efficacy of nine fungicides was tested with one untreated control. The fungicide solutions were prepared by dissolving known quantity of fungicide in water to get desired concentration. First spray was applied on the appearance of the disease initiation.

3.3.1 Design of the layout

The experiment was laid out in a completely randomized block design with three replications. The treatments were randomly allotted to plots. The details are as follows.

Treatments	: 10
Number of plants tagged/treatment	: 5
Spacing	: 30 cm X 10 cm
Plot size	: 03 m X 03 m = 09 Sq. m
Variety	: JG11
Date of sowing	: October 10, 2015.

Treatments details



R-I	R-II	R-III
T_1	T_9	T_{10}
T_8	T_6	T_9
T_5	T_2	T_6
T_7	T_3	T_8
T_4	T_1	T_5
T_2	T_{10}	T_3
T_6	T_5	T_1
T_9	T_7	T_4
T_3	T_8	T_2
T_{10}	T_4	T_7

3.3.2 Observations recorded

Powdery mildew on chickpea was recorded by using 0-5 scale and Percent Disease Index (PDI) was calculated by using the formula given by Wheeler (1969).

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Total number of leaves observed x maximum grade}} \times 100$$

3.3.3 Yield

All the treatments were harvested separately and average weight was calculated. After threshing and winnowing seed weight of each replication in kilogram was recorded and yield per hectare was computed by using net plot yield data and it was then converted to quintals per hectare.

3.3.4 Statistical analysis

The statistical analysis of completely randomized block design was carried out as per the procedure given by Panse and Sukhatme (1985). Per cent data were transformed to arc sine values and analyzed statistically.

3.3.5 Benefit Cost Ratio (BCR)

Total cost incurred for application of fungicides including cost of fungicides and labours were calculated. Additional benefit due to increased yield in each treatment over control was worked out and benefit cost ratio was calculated using additional benefits and total costs.

4. EXPERIMENTAL RESULTS

The results of the investigation on biochemical, nutrient status and field evaluation of new molecules of fungicides associated with the resistance to powdery mildew of chickpea caused by *Leveillula taurica* (Lev.) Arnaud conducted during 2015-2016 are presented as here under.

4.1 Biochemical parameters in chickpea

4.1.1 Chlorophyll

The chlorophyll content in infected and healthy leaves were analysed from 63 to 91 Days after sowing (DAS) at an interval of a week, the results of chlorophyll-a, chlorophyll-b and total chlorophyll contents are represented in (Table 1, Fig 1).

Results from the Table 1 revealed that the chlorophyll (a, b and total) was high in healthy leaves of chickpea variety JG-11 tested at 63, 70, 77, 84 and 91 DAS and the total mean value was 0.626 mg g^{-1} as compared to diseased leaves with a value of 0.462 mg g^{-1}. As the days advance there was a gradual decrease in chlorophyll content in both healthy and diseased leaves.

At 63 DAS, the chlorophyll content greatly differed between healthy and diseased leaf sample. In general it was found higher in healthy than powdery mildew infected leaves. The values are 1.06 mg g^{-1} and 0.91 mg g^{-1} of chlorophyll-a, 0.49 mg g^{-1} and 0.26 mg g^{-1} of chlorophyll-b and 1.55 mg g^{-1} and 1.17 mg g^{-1} of total chlorophyll in healthy and diseased leaf sample respectively and recorded a mean of 1.03 mg g^{-1} and 0.78 mg g^{-1} in healthy and diseased leaf sample respectively.

At 70 DAS chlorophyll content was reduced gradually both in infected and healthy leaf sample. Chlorophyll-a content was 0.95 mg g^{-1} and 0.88 mg g^{-1}, chlorophyll-b content was 0.33 mg g^{-1} and 0.21 mg g^{-1} and total chlorophyll content was 1.28 mg g^{-1} and 1.09 mg g^{-1} in healthy and diseased leaf sample respectively and recorded mean of 0.85 mg g^{-1} and 0.72 mg g^{-1} in healthy and diseased leaf sample respectively.

At 77 DAS chlorophyll content was reduced both in infected and healthy leaf sample. Chlorophyll-a content was 0.88 mg g^{-1} and 0.64 mg g^{-1}, chlorophyll-b content was 0.29 mg g^{-1} and 0.19 mg g^{-1} and total chlorophyll content was 1.16 mg g^{-1} and 0.83 mg g^{-1} in healthy and diseased leaf sample respectively and recorded mean of 0.77 mg g^{-1} and 0.55 mg g^{-1} in healthy and diseased leaf sample respectively.

At 84 DAS chlorophyll content was reduced both in infected and healthy leaf sample. Chlorophyll-a content was 0.62 mg g^{-1} and 0.22 mg g^{-1}, chlorophyll-b content was 0.14 mg g^{-1} and 0.04 mg g^{-1} and total chlorophyll content was 0.66 mg g^{-1} and 0.36 mg g^{-1} in healthy and diseased leaf sample respectively and recorded mean of 0.44 mg g^{-1} and 0.24 mg g^{-1} in healthy and diseased leaf sample respectively.

At 91 DAS chlorophyll content was reduced both in infected and healthy leaf sample. Chlorophyll-a content was 0.02 mg g^{-1} and 0.01 mg g^{-1}, chlorophyll-b content was 0.02 mg g^{-1} and 0.00 mg g^{-1} and total chlorophyll content was 0.04 mg g^{-1} and 0.02 mg g^{-1} in healthy and diseased leaf sample respectively and recorded mean of 0.02 mg g^{-1} and 0.01 mg g^{-1} in healthy and diseased leaf sample respectively.

General view of the plots

Pestle and morter for grinding

Diseased leaves

Healthy leaves

Plate 1: General view of the field with healthy and diseased leaf samples.

Table 1. Chlorophyll (a, b and Total) content (mg g^{-1} of fresh weight) in healthy and diseased leaves of chickpea powdery mildew.

	Chlorophyll (mg/g)											
	63 DAS		70 DAS		77 DAS		84 DAS		91 DAS		Mean	
Chloro Phyll	H	D	H	D	H	D	H	D	H	D	H	D
A	1.06	0.91	0.95	0.88	0.88	0.64	0.62	0.22	0.02	0.01	0.706	0.532
B	0.49	0.26	0.33	0.21	0.29	0.19	0.14	0.04	0.02	0.00	0.234	0.16
Total	1.55	1.17	1.28	1.09	1.16	0.83	0.66	0.36	0.04	0.02	0.938	0.694
Mean	1.03	0.78	0.85	0.72	0.77	0.55	0.44	0.24	0.02	0.01	0.626	0.462

DAS- Days After Sowing

H – Healthy

D – Diseased

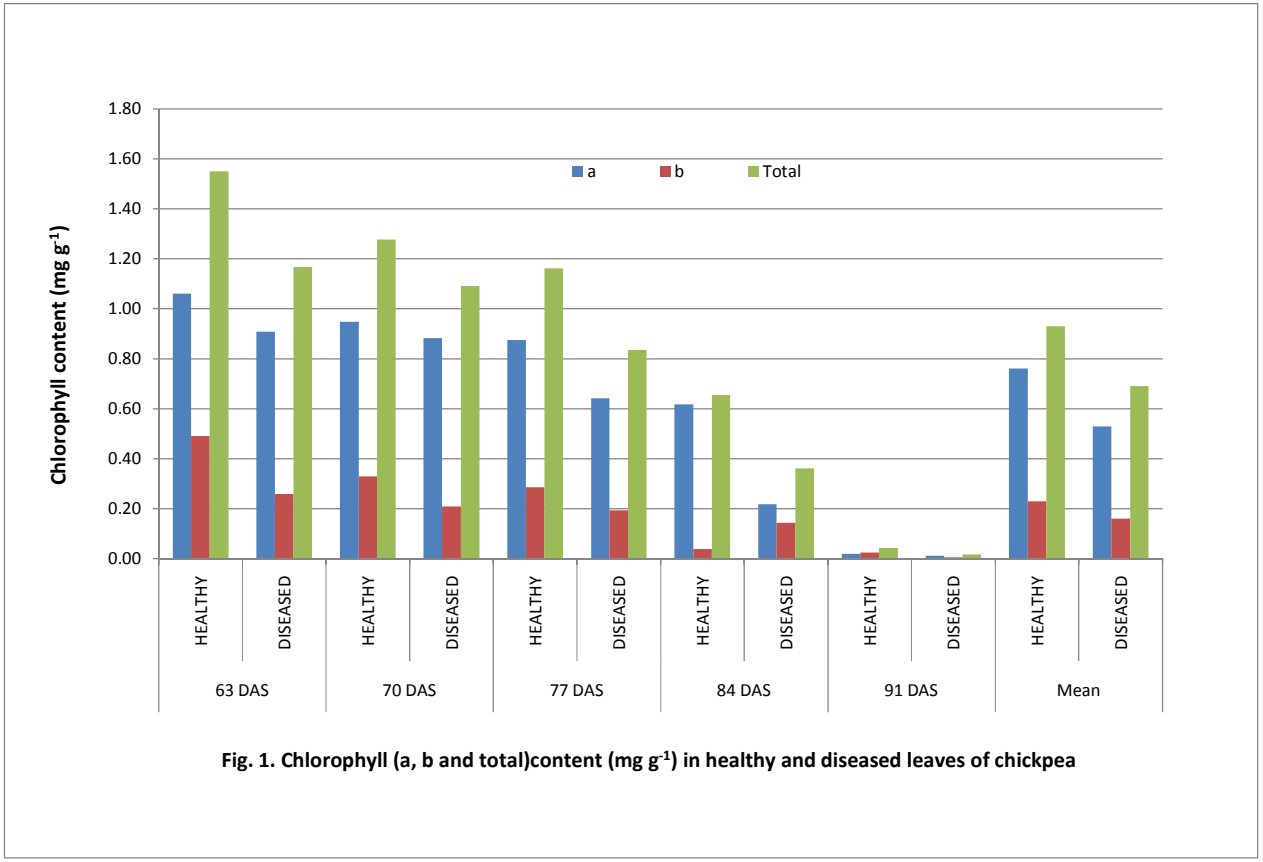


Fig. 1. Chlorophyll (a, b and total)content (mg g^{-1}) in healthy and diseased leaves of chickpea

Fig 1: Chlorophyll (a, b and total) content (mg g^{-1}) in healthy and diseased leaves of chickpea

4.1.2 Total phenol

The results of total phenol estimated in healthy and powdery mildew infected leaves of chickpea variety JG-11 are presented in Table 2 and Fig 2, total phenol estimated at 63, 70, 77, 84 and 91 DAS and the total phenol was more in healthy leaves as compared to diseased leaves. As the days advanced there was a gradual increase in total phenol content in both healthy and diseased leaves and found highest at 91 DAS (at harvest stage) with 3.87 mg g^{-1} and 3.08 mg g^{-1} in healthy and diseased leaves respectively.

The total phenol content was more in healthy leaves than diseased leaves and it gradually increased from 63 DAS (3.12 mg g^{-1} , 1.67 mg g^{-1}) , 70 DAS (3.31 mg g^{-1} , 1.70 mg g^{-1}), 77 DAS (3.57 mg g^{-1} , 1.87 mg g^{-1}), 84 DAS (3.73 mg g^{-1} , 2.00 mg g^{-1}) and 91 DAS (3.87 mg g^{-1} , 3.08 mg g^{-1}) in healthy and diseased leaves respectively.

4.1.3 Sugars

The sugar content was analysed from healthy and powdery mildew infected leaves of chickpea samples collected at 63 to 91 DAS at an interval of a week and the results of reducing, non-reducing and total sugar contents are represented in (Table 3, Fig 3).

The results in Table 3 revealed that the sugar (reducing, non-reducing and total sugar) content was high in healthy leaves of chickpea variety JG-11 tested at 63, 70, 77, 84 and 91 DAS and the total mean value was 2.57 mg g^{-1} in healthy leaves as compared to diseased leaves with a value of 2.20 mg g^{-1}. As the days advanced there was a gradual decrease in sugar content in both healthy and diseased leaves.

At 63 DAS, the sugar content greatly differed between healthy and diseased leaf sample. In general it found higher in healthy than powdery mildew infected leaf sample. The values are 3.31 mg g^{-1} and 2.43 mg g^{-1} of reducing sugar, 1.88 mg g^{-1} and 1.97 mg g^{-1} of non-reducing sugar and 5.18 mg g^{-1} and 4.40 mg g^{-1} of total sugar in healthy and diseased leaf sample respectively. Mean of sugar content was 3.46 mg g^{-1} and 2.93 mg g^{-1} in healthy and diseased leaf sample respectively.

At 70 DAS, the sugar content greatly differed between healthy and diseased leaf sample. In general it found higher in healthy than powdery mildew infected leaf sample. The values are 2.52 mg g^{-1} and 2.31 mg g^{-1} of reducing sugar, 2.17 mg g^{-1} and 1.89 mg g^{-1} of non-reducing sugar and 4.68 mg g^{-1} and 4.20 mg g^{-1} of total sugar in healthy and diseased leaf sample respectively. Mean of sugar content was 3.12 mg g^{-1} and 2.80 mg g^{-1} in healthy and diseased leaf sample respectively.

At 77 DAS, the sugar content differed between healthy and diseased leaf sample. In general it found higher in healthy than powdery mildew infected leaf sample. The values are 2.20 mg g^{-1} and 1.12 mg g^{-1} of reducing sugar, 1.17 mg g^{-1} and 1.51 mg g^{-1} of non-reducing sugar and 3.93 mg g^{-1} and 3.63 mg g^{-1} of total sugar in healthy and diseased leaf sample respectively. Mean of sugar content was 2.63 mg g^{-1} and 2.09 mg g^{-1} in healthy and diseased leaf sample respectively.

Plate 2: Plant extract and it's crude sample

Table 2. Phenol content (mg g^{-1} of fresh weight) in healthy and diseased leaves of chickpea powdery mildew.

	Total phenols (mg/g)											
	63 DAS		70 DAS		77 DAS		84 DAS		91 DAS		Mean	
	H	D	H	D	H	D	H	D	H	D	H	D
1	3.12	1.67	3.31	1.70	3.57	1.87	3.73	2.00	3.87	3.08	3.52	2.06

DAS- Days After Sowing

H – Healthy

D – Diseased

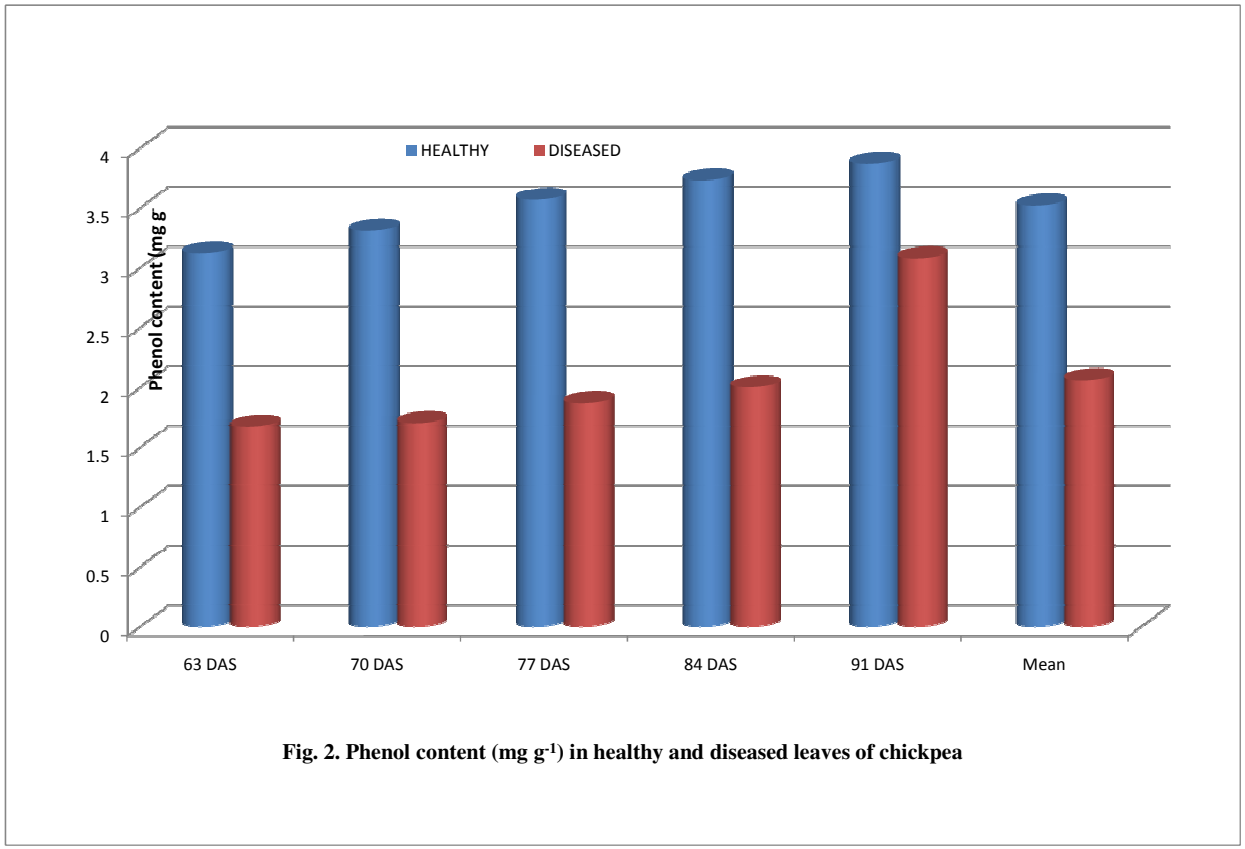


Fig. 2. Phenol content (mg g^{-1}) in healthy and diseased leaves of chickpea

Fig 2: Phenol content (mg g^{-1}) in healthy and diseased leaves of chickpea

Table 3. Sugar (reducing, non-reducing and total sugar) content (mg g^{-1} of fresh weight) in healthy and diseased leaves of chickpea powdery mildew.

	Sugars (mg/g)											
	63 DAS		70 DAS		77 DAS		84 DAS		91 DAS		Mean	
	H	D	H	D	H	D	H	D	H	D	H	D
Reducing sugar	3.31	2.43	2.52	2.31	2.20	1.12	2.01	1.99	1.20	1.04	2.24	1.78
Non reducing	1.88	1.97	2.17	1.89	1.77	1.51	1.59	1.13	0.65	0.60	1.61	1.42
Total sugar	5.18	4.40	4.68	4.20	3.93	3.63	3.60	3.12	1.85	1.63	3.85	3.40
Mean	3.46	2.93	3.12	2.8	2.63	2.09	2.40	2.08	1.23	1.09	2.57	2.20

DAS- Days After Sowing

H – Healthy

D – Diseased

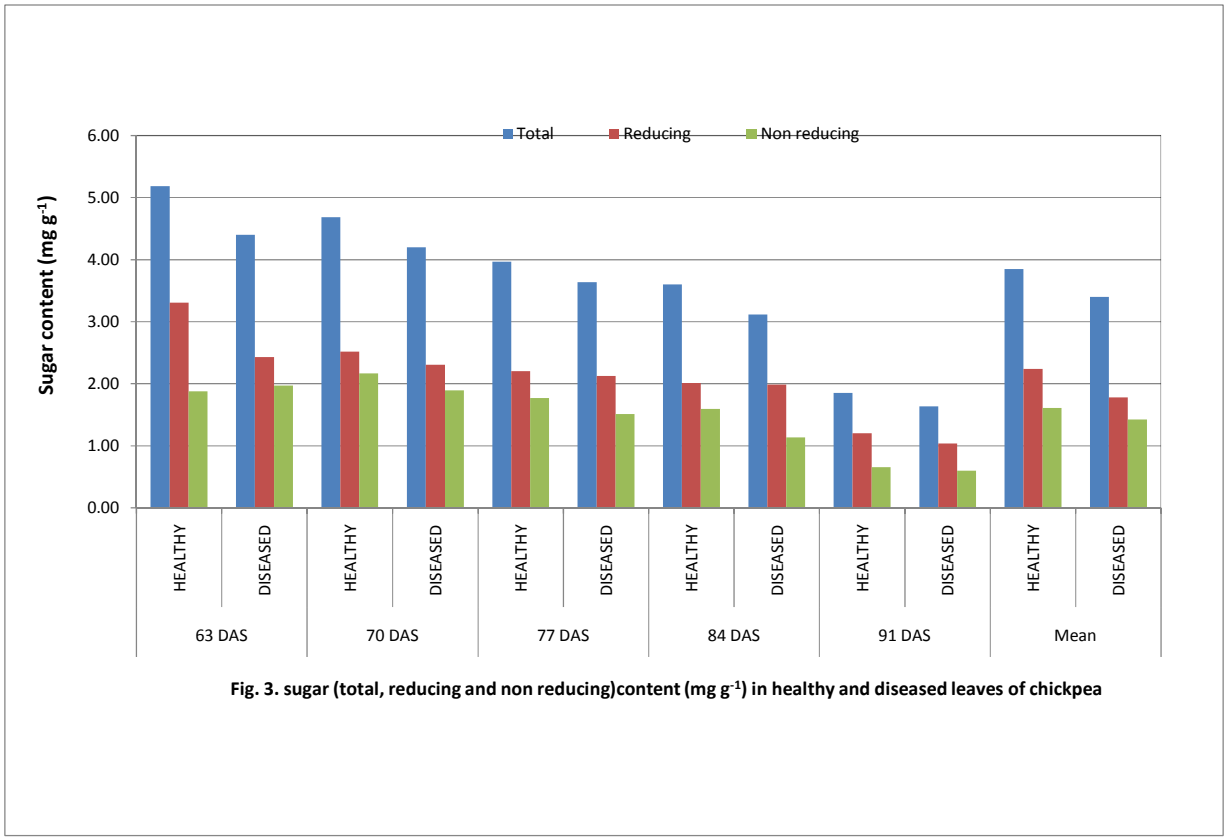


Fig. 3. sugar (total, reducing and non reducing)content (mg g^{-1}) in healthy and diseased leaves of chickpea

Fig 3: Sugar (total, reducing and non reducing)content (mg g^{-1}) in healthy and diseased leaves of chickpea

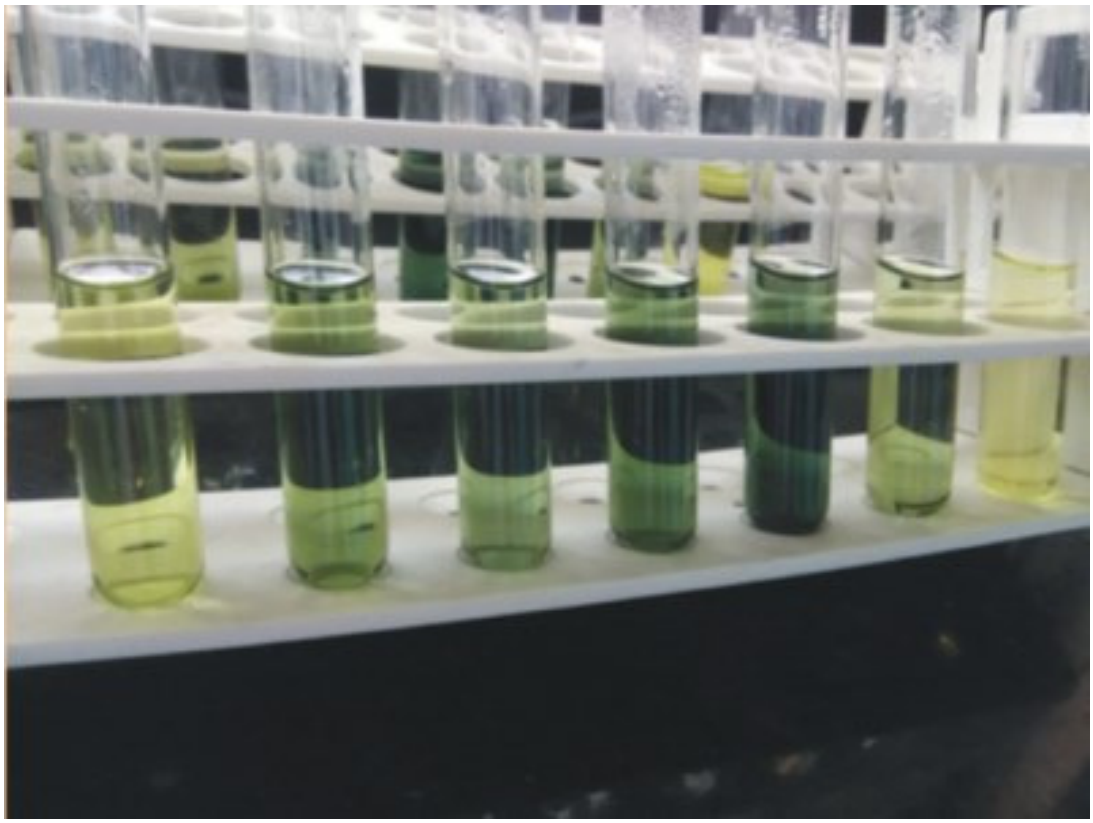


Plate 3: Test tubes containg plant extract for analysis

At 84 DAS, the sugar content differed between healthy and diseased leaf sample. In general it found higher in healthy than powdery mildew infected leaf sample. The values are 2.01 mg g^{-1} and 1.99 mg g^{-1} of reducing sugar, 1.59 mg g^{-1} and 1.13 mg g^{-1} of non-reducing sugar and 3.60 mg g^{-1} and 3.12 mg g^{-1} of total sugar in healthy and diseased leaf sample respectively. Mean of sugar content was 2.40 mg g^{-1} and 2.08 mg g^{-1} in healthy and diseased leaf sample respectively.

At 91 DAS, means at harvest stage of the crop, where the plants are completely dried in the field itself. The sugar content differed between healthy and diseased leaf sample. In general sugar content found higher in healthy than powdery mildew infected leaf sample. The values are 1.20 mg g^{-1} and 1.04 mg g^{-1} of reducing sugar, 0.65 mg g^{-1} and 0.60 mg g^{-1} of non-reducing sugar and 1.85 mg g^{-1} and 1.63 mg g^{-1} of total sugar in healthy and diseased leaf sample respectively. Mean of sugar content was 1.23 mg g^{-1} and 1.09 mg g^{-1} in healthy and diseased leaf sample respectively.

Mean reducing sugar in healthy and infected leaves was 2.24 mg g^{-1} and 1.78 mg g^{-1} respectively, mean non reducing sugar was 1.61 mg g^{-1} and 1.42 mg g^{-1} in healthy and powdery mildew infected leaves respectively. Total sugar was 3.85 mg g^{-1} and 3.40 mg g^{-1} in healthy and infected leaves respectively.

4.1.4 Proteins

The protein content estimated in healthy and infected leaves of chickpea variety JG-11 is presented in Table 4 and Fig 4, protein was estimated at 63, 70, 77, 84 and 91 DAS and the total mean protein was 6.33 mg g^{-1} in healthy leaves as compared to diseased leaves with a value of 5.04 mg g^{-1}. As the days advance gradual decrease in protein content in both healthy and diseased leaves were recorded.

The protein content was more in healthy leaves compared to diseased leaves. Protein content was gradually decreased at 63 DAS (7.36 mg g^{-1}, 6.20 mg g^{-1}), 70 DAS (7.08 mg g^{-1} , 5.84 mg g^{-1}), 77 DAS (6.87 mg g^{-1} , 5.36 mg g^{-1}), 84 DAS (5.77 mg g^{-1} , 4.28 mg g^{-1}) and 91 DAS (4.60 mg g^{-1}, 3.53 mg g^{-1}) in healthy and diseased leaves respectively.

4.1.5 Amino acids

Amino acid content in healthy and diseased leaves of chickpea variety JG-11 was assessed and the results are presented in Table 5 and Fig 5, amino acid content was assessed regularly at 63, 70, 77, 84 and 91 DAS and the total mean amino acid content was 1.12 mg g^{-1} as compared to diseased leaves with a value of 0.74 mg g^{-1}. As the days advance there was a gradual decrease in total amino acid content in both healthy and diseased leaves and found highest at 63 DAS (9^{th} week) with 2.07 mg g^{-1} and 1.63 mg g^{-1} in healthy and diseased leaves respectively.

The amino acid content observed more in healthy leaves than diseased leaves and it gradually decreased at 63 DAS (2.07 mg g^{-1} , 1.63 mg g^{-1}) , 70 DAS (1.57 mg g^{-1} , 1.02 mg g^{-1}), 77 DAS (0.72 mg g^{-1} , 0.40 mg g^{-1}), 84 DAS (0.67 mg g^{-1} , 0.37 mg g^{-1}) and 91 DAS (0.60 mg g^{-1} , 0.30 mg g^{-1}) in healthy and diseased leaves respectively.

Table 4. Protein content (mg g^{-1} of fresh weight) in healthy and diseased leaves of chickpea powdery mildew.

	Protein (mg/g)											
	63 DAS		70 DAS		77 DAS		84 DAS		91 DAS		Mean	
	H	D	H	D	H	D	H	D	H	D	H	D
1	7.36	6.20	7.08	5.84	6.87	5.36	5.77	4.28	4.60	3.53	6.33	5.04

DAS- Days After Sowing

H – Healthy

D – Diseased

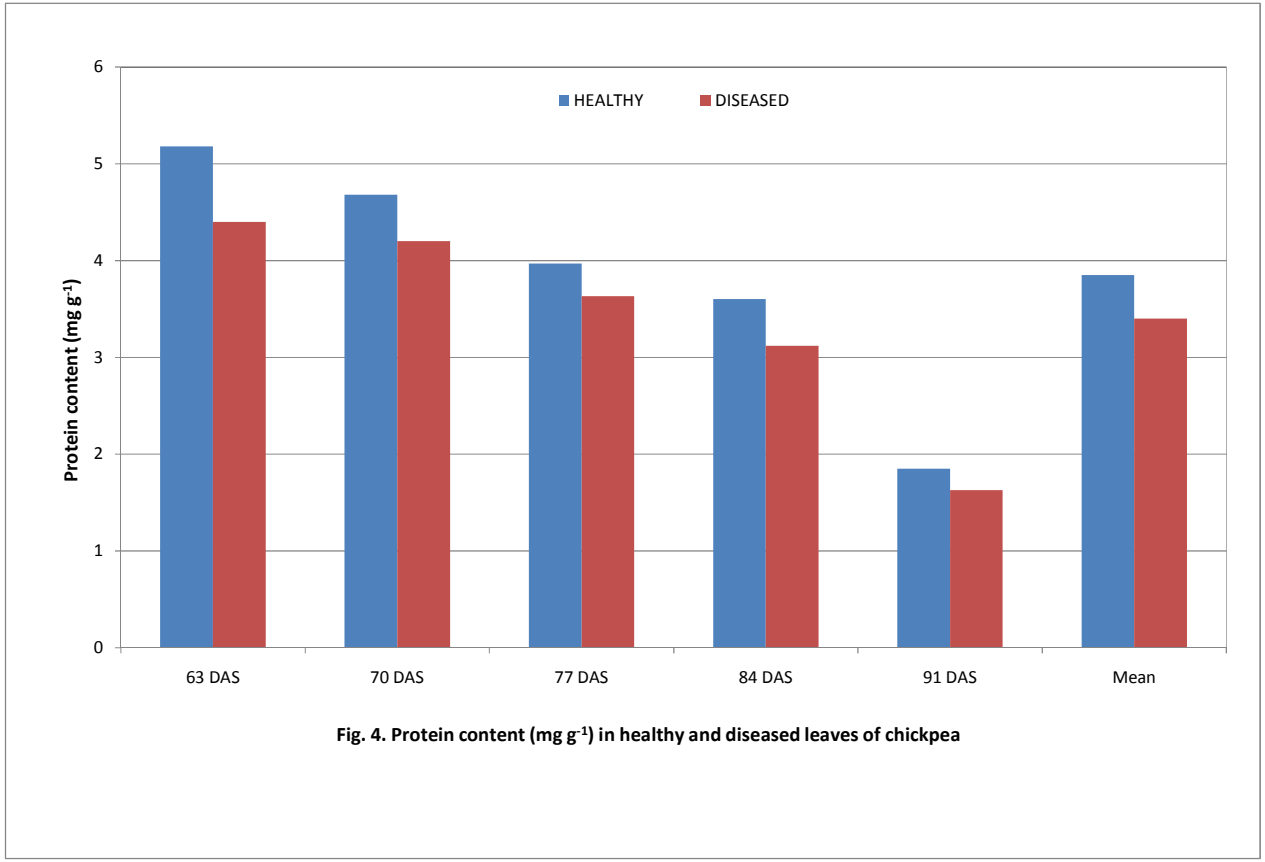


Fig. 4. Protein content (mg g^{-1}) in healthy and diseased leaves of chickpea

Fig 4: Protein content (mg g^{-1}) in healthy and diseased leaves of chickpea

Table 5. Amino acid content (mg g^{-1} of fresh weight) in healthy and diseased leaves of chickpea powdery mildew.

	Amino Acid (mg/g)											
	63 DAS		70 DAS		77 DAS		84 DAS		91 DAS		Mean	
	H	D	H	D	H	D	H	D	H	D	H	D
1	2.07	1.63	1.57	1.02	0.72	0.40	0.67	0.37	0.60	0.30	1.12	0.74

DAS- Days After Sowing

H – Healthy

D – Diseased

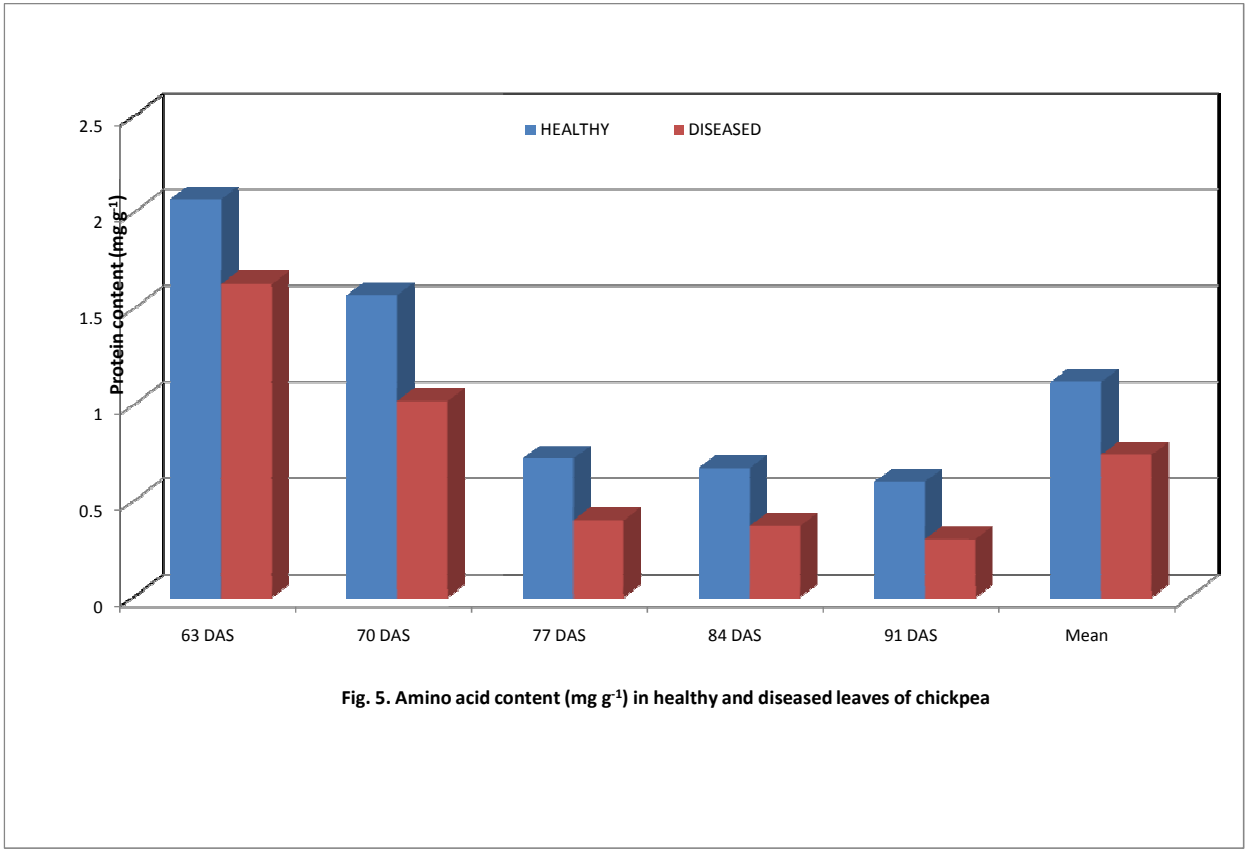


Fig. 5. Amino acid content (mg g^{-1}) in healthy and diseased leaves of chickpea

Fig 5: Amino acid content (mg g^{-1}) in healthy and diseased leaves of chickpea

Plate 4: Healthy and diseased plants

4.2 Nutrient parameters of chickpea

4.2.1 Minerals

An attempt was made to know the effect of powdery mildew infection on mineral nutrition in chickpea. The leaf sample analysis was done with an interval of a week after the disease development and the last analysis was carried out during the harvest stage.

The mineral content in healthy and diseased leaves of chickpea variety JG-11 was estimated and results are presented in Table 6, minerals like N, P, K, Mg, Zn, Fe, Mn, Cu, and B were analysed at 63, 70, 77, 84 and 91 DAS in both diseased and healthy leaves.

The nitrogen content was analysed and it was found highest in healthy leaves than diseased leaves and it gradually decreased from 63 DAS (3.48 %, 2.60 %), 70 DAS (3.40 %, 2.57 %), 77 DAS (2.79 %, 2.56 %), 84 DAS (2.50 %, 2.41 %) and 91 DAS (2.25 %, 1.99 %) in healthy and diseased leaves respectively. The total mean per cent N content was more in healthy leaves compared to diseased leaves (Fig 6).

The phosphorous content was found highest in healthy leaves than infected leaves and it was gradually increased in healthy leaf sample and vice versa in diseased sample. Observations were recorded at 63 DAS (0.134 %, 0.115 %), 70 DAS (0.146 %, 0.112 %), 77 DAS (0.152 %, 0.110 %), 84 DAS (0.162 %, 0.104 %) and 91 DAS (0.164 %, 0.099 %) from healthy and diseased leaves respectively. The total mean per cent P was 0.151 % in healthy leaves as compared to diseased leaves containg 0.108 % (Fig 6).

The per cent potash content was found highest in healthy leaves than infected leaves and it gradually increased in both healthy and diseased samples. Observation recorded at 63 DAS (1.26 %, 1.01 %), 70 DAS (1.37 %, 1.08 %), 77 DAS (1.89 %, 1.44 %), 84 DAS (1.98 %, 1.67 %) and 91 DAS (2.28 %, 1.69 %) in healthy and diseased leaves respectively. The total mean per cent K was 1.75 % as compared to diseased leaves 1.37 % (Fig 6).

The per cent magnesium content was found highest in healthy leaves than infected leaves and it gradually increased in both healthy and diseased samples. Observation recorded at 63 DAS (0.24 %, 0.16 %), 70 DAS (0.36 %, 0.30 %), 77 DAS (0.62 %, 0.40 %), 84 DAS (0.84 %, 0.48 %) and 91 DAS (1.32 %, 0.54 %) in healthy and diseased leaves respectively. The total mean per cent Mg content was more in healthy leaves 0.67 % as compared to diseased leaves 0.37 % (Fig 6).

The per cent zinc content was found highest in healthy leaves than infected leaves and it gradually increased in both healthy and diseased samples. Observation recorded at 63 DAS (28.2 ppm, 18.1 ppm), 70 DAS (31.4 ppm, 20.8 ppm), 77 DAS (52 ppm, 42.9 ppm), 84 DAS (58.9 ppm, 48.8 ppm) and 91 DAS (62.6 ppm, 53.7 ppm) in healthy and diseased leaves respectively. The total mean per cent Zn was 46.62 ppm as compared to diseased leaves 36.86 ppm (Fig 8).

The per cent iron content was found highest in infected leaves than healthy leaves and it gradually increased in both healthy and diseased samples. Observation recorded at 63 DAS (410 ppm, 579 ppm), 70 DAS (650 ppm, 688 ppm), 77 DAS (668 ppm, 1501 ppm), 84 DAS (1434 ppm, 1688 ppm) and 91 DAS (1598 ppm, 1983 ppm) in healthy and diseased leaves respectively. The total mean per cent Fe content was more in healthy leaves 632 ppm as compared to diseased leaves 1287 ppm (Fig 7).

Table 6. Quantitative status of nutrients content in healthy and diseased leaves of chickpea infected with powdery mildew.

	63 DAS		70 DAS		77 DAS		84 DAS		91 DAS		Mean	
MINARALS	H	D	H	D	H	D	H	D	H	D	H	D
N(%)	3.48	2.60	3.40	2.57	2.79	2.56	2.50	2.41	2.25	1.99	2.88	2.42
P(%)	0.13	0.12	0.15	0.11	0.15	0.11	0.16	0.10	0.16	0.10	0.15	0.10
K(%)	1.26	1.01	1.37	1.08	1.89	1.44	1.98	1.67	2.28	1.69	1.75	1.37
Mg(%)	0.24	0.16	0.36	0.30	0.62	0.40	0.84	0.48	1.32	0.54	0.67	0.37
Zn(ppm)	28.2	18.1	31.4	20.8	52	42.9	58.9	48.8	62.6	53.7	46.6	36.9
Fe(ppm)	410	579	650	688	668	1501	1434	1688	1598	1983	632	1287
Mn(ppm)	223.2	214.9	323.8	200.2	326.0	168.8	367.6	139.6	368.4	103.3	321.8	165.3
Cu(ppm)	20.2	16.3	19.4	14.7	9.8	4.4	8.7	3.9	8.7	2.8	13.36	8.42
B(ppm)	9.98	10.25	10.08	12.23	10.43	12.30	11.15	12.76	15.9	26.8	11.50	14.86

DAS- Days After Sowing
H – Healthy
D – Diseased

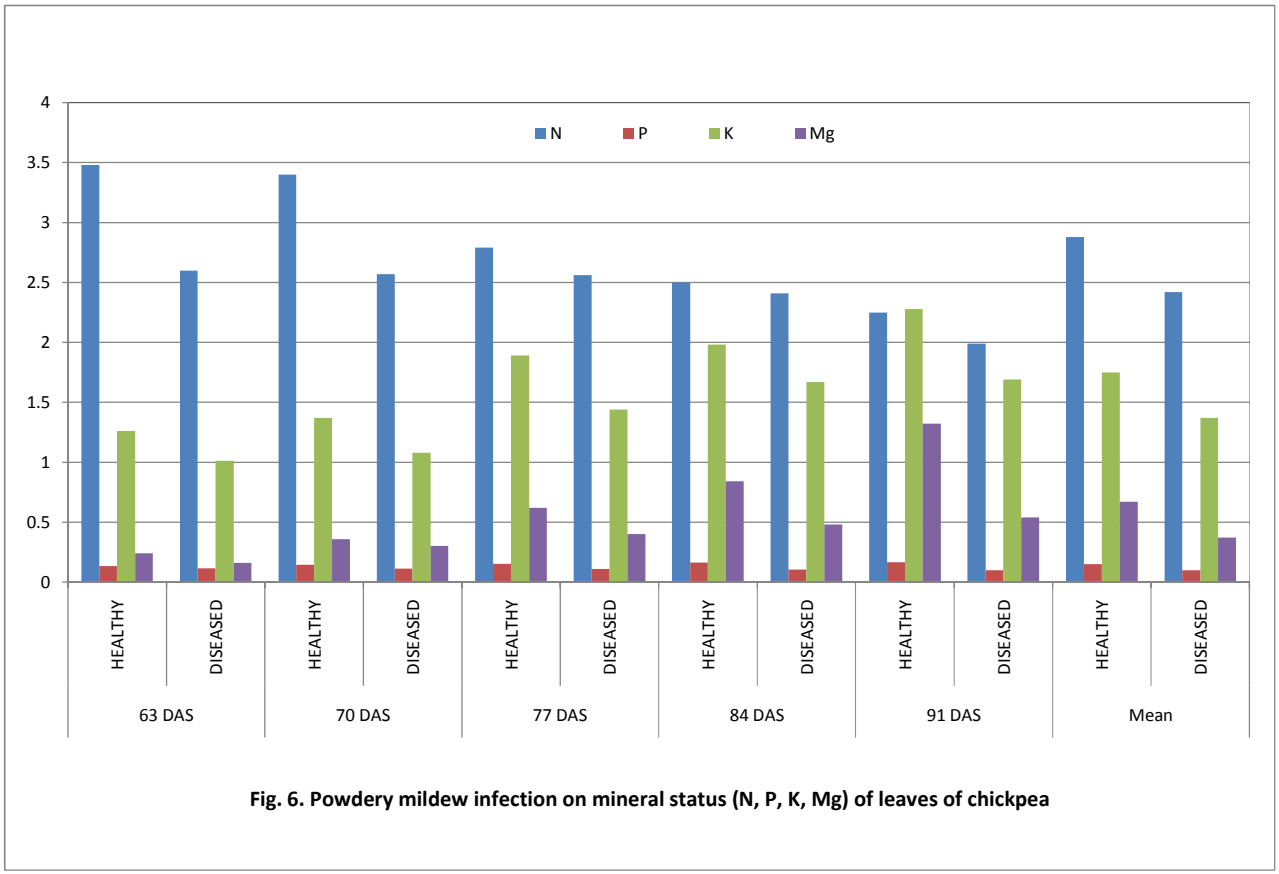


Fig. 6. Powdery mildew infection on mineral status (N, P, K, Mg) of leaves of chickpea

Fig 6: Powdery mildew infection on mineral status (N, P, K, Mg) of leaves of chickpea

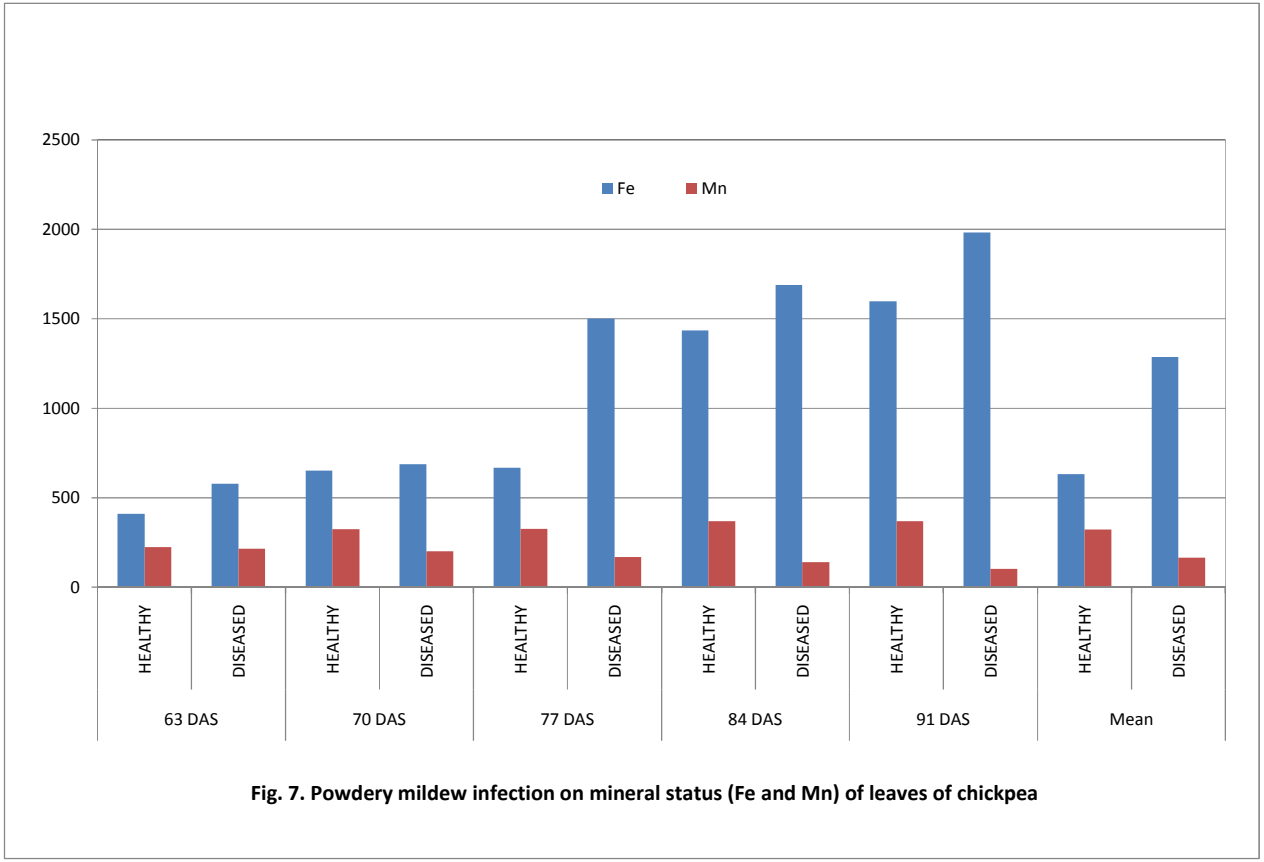


Fig. 7. Powdery mildew infection on mineral status (Fe and Mn) of leaves of chickpea

Fig 7: Powdery mildew infection on mineral status (Fe and Mn) of leaves of chickpea

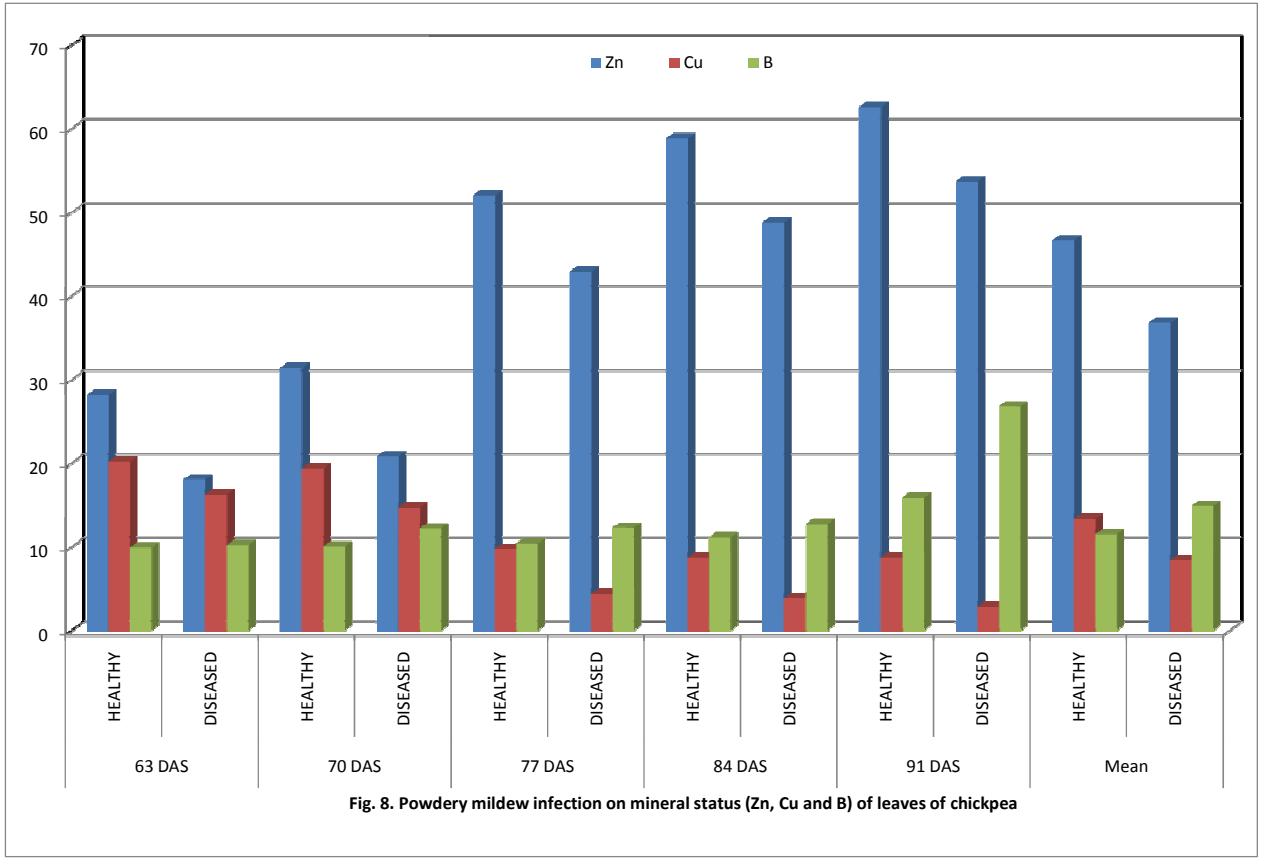


Fig. 8. Powdery mildew infection on mineral status (Zn, Cu and B) of leaves of chickpea

Fig 8: Powdery mildew infection on mineral status (Zn, Cu and B) of leaves of chickpea

Plate 5: General view of the field for fungicide evaluation

Table 7: Efficacy of fungicides against chickpea powdery mildew during *rabi* 2015 at ARS Annigeri.

Sl No.	Treatment	Concentration (%)	PDI	Yield (Q/ha)	Gross return	Cost of cultivation	Net return	B:C ratio
1	Wettable Sulphur 80 % WP	0.3	08.89 (17.26)*	12.42	68310	22088	46222	1.21
2	Carbendazim 50 % WP	0.1	07.41 (15.76)	12.44	68420	23183	45237	1.18
3	Difenconazole 25 % EC	0.05	02.22 (08.57)	14.07	77385	24196	53190	1.39
4	Azoxystrobin 23 % EC	0.05	00.00 (00.00)	14.09	77495	25321	52175	1.37
5	Tebuconazole 25.9 % EC	0.1	02.22 (08.57)	13.96	76780	24473	52307	1.37
6	Hexaconazole 5 % EC	0.1	02.96 (09.77)	13.76	75680	22403	53277	1.39
7	Propiconazole 25 % EC	0.1	02.96 (09.77)	13.80	75900	23633	52267	1.37
8	Myclobutanil 10 % WP	0.2	00.00 (00.00)	14.20	78100	21734	56366	1.48
9	Captan 70 % + Hexaconazole 5 % WP	0.2	08.15 (16.55)	12.78	70290	25133	45157	1.18
10	Control	-	43.70 (41.37)	10.89	59895	21683	38212	-
SEm±			0.98	0.64				
CD at 5%			2.91	1.98				

*Figures in the parenthesis are arc sine transformed values.

It is calculated by assuming market rate of chickpea variety JG 11 as Rs. 2125/q.

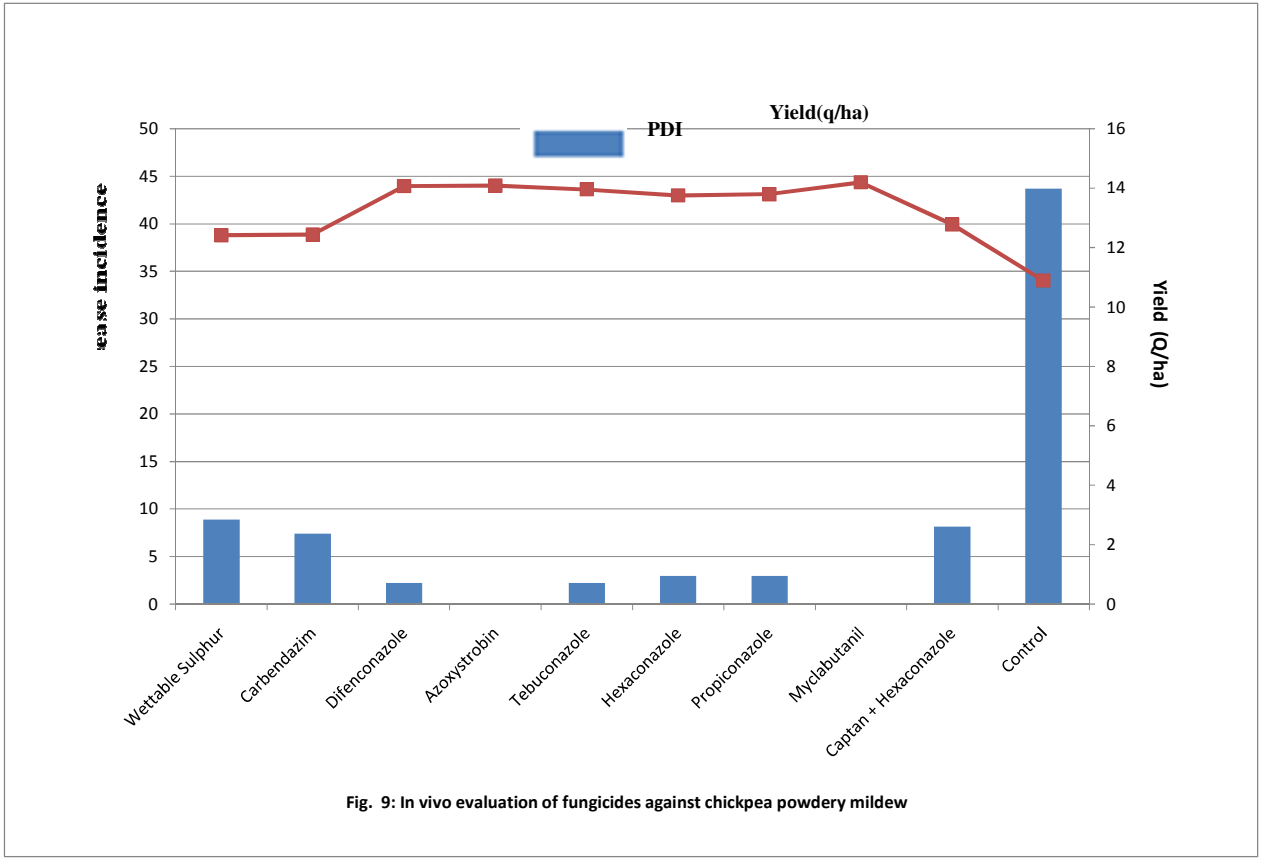


Fig. 9: In vivo evaluation of fungicides against chickpea powdery mildew

Fig 9: *In vivo* evaluation of fungicides against chickpea powdery mildew

The per cent manganese content was found highest in healthy leaves than infected leaves and it gradually increased at healthy leaf sample and vice versa in infected leaf sample. Observation recorded at 63 DAS (223.2 ppm, 214.9 ppm), 70 DAS (323.8 ppm, 200.2 ppm), 77 DAS (326 ppm, 168.8 ppm), 84 DAS (367.6 ppm, 139.6 ppm) and 91 DAS (368.4 ppm, 103.3 ppm) in healthy and diseased leaves respectively. The total mean per cent Mn was 321.8 ppm as compared to diseased leaves 165.3 ppm (Fig 7).

The per cent copper content was found highest in healthy leaves than infected leaves and it gradually decreased in both health and powdery mildew infected leaf sample. Observations were recorded at 63 DAS (20.2 ppm, 16.3 ppm), 70 DAS (19.4 ppm, 14.7 ppm), 77 DAS (9.8 ppm, 4.4 ppm), 84 DAS (8.7 ppm, 3.9 ppm) and 91 DAS (8.7 ppm, 2.8 ppm) in healthy and diseased leaves respectively. The total mean per cent Cu was 13.36 ppm as compared to diseased leaves 8.42 ppm (Fig 8).

The per cent boron content was found highest in diseased leaves than healthy leaves and it gradually increased in both healthy and powdery mildew infected leaf sample. Observation recorded at 63 DAS (9.98 ppm, 10.25 ppm), 70 DAS (10.08 ppm, 12.23 ppm), 77 DAS (10.43 ppm, 12.30 ppm), 84 DAS (11.15 ppm, 12.76 ppm) and 91 DAS (15.9 ppm, 26.8 ppm) in healthy and diseased leaves respectively. The total mean per cent B was 11.50 ppm as compared to diseased leaves 14.86 ppm (Fig 8).

4.3 Field evaluation of fungicides

Field experiment was conducted to evaluate the relative efficacy of fungicides against powdery mildew of chickpea during *Rabi* 2015-16 with nine fungicides *viz.,* Wettable sulphur (0.3 %), carbendazim (0.1 %), difenconazole (0.05 %), azoxystrobin (0.05 %), tebuconazole (0.1 %), hexaconazole (0.1 %), propiconazole (0.1 %), myclobutanil (0.2 %) and captan + hexaconazole (0.2 %) along with control. Percent Disease Index (PDI), per cent reduction of disease over control and yield (q/ha) were recorded and the results are presented in Table 7, Fig 9.

From the Table 7 it is very clear that all the treatments reduced the disease significantly compared to the unprotected plot. Among different fungicides myclobutanil and azoxystrobin reduced disease cent per cent and significantly superior over all other treatments followed by difenconazole (2.22 PDI), tebuconazole (2.22 PDI) hexaconazole (2.96 PDI) and propiconazole (2.96 PDI) which were at par with each other and significantly superior over control. Wettable sulphur (08.89 PDI) was least effective and disease severity in control was highest (43.70 PDI).

It is also clear that myclobutanil and azoxystrobin found to be superior over the other treatments. With respect to yield and all other treatments were at par with each other. Highest yield was recorded in myclobutanil (0.2 %) followed by azoxystrobin (0.05 %) recording 14.20 q/ha and 14.09 q/ha yield respectively. Difenconazole, tebuconazole, propiconazole, and hexaconazole were found at par with each other. Among the fungicidal treatments, minimum yield was obtained in wettable sulphur (12.42 q/ha) followed by carbendazim (12.44 q/ha). Lowest yield was recorded in unsprayed treatment (10.89 q/ha).

Highest B:C ratio was found in myclobutanil (1.48) fallowed by difenconazole (1.39) and hexaconazole (1.39).

5. DISCUSSION

Chickpea powdery mildew is a foliar disease not known to cause a widespread damage in chickpea as like other diseases. In India appearance of powdery mildew on chickpea was first reported by Mandhare *et al*. (2005) from Maharashtra on chickpea cultivar. Visual incidence was observed from flowering to podding. In Karnataka Muhammad *et al.* (2012) reported 30 to 40 Per cent incidence in RARS, Vijayapur, and 25 to 30 per cent incidence in farmers' field in Hiriyur.

To elucidate the biochemical and nutrient status between the healthy and diseased leaves and to evaluate new fungicide molecules under natural epiphytotic condition to study the relative efficacy against the powdery mildew in chickpea variety JG-11. The results of the present investigations of powdery mildew of chickpea caused by *Leveillula taurica* are discussed here under.

5.1 Biochemical parameters in chickpea

In recent years, it is becoming increasingly evident that several natural and induced defense mechanisms operate in host plants against different diseases. One such defense mechanism is the presence of certain compounds inhibitory to the pathogen. Sometimes, the host plant is induced to synthesize these compounds on infection. Analysis of biochemicals in healthy and diseased leaves was carried out to understand the role of these biochemicals in infection in chickpea.

5.1.1 Chlorophyll content

In the current investigation of chlorophyll-a, chlorophyll-b and total chlorophyll content was recorded in both healthy and infected leaves at 63, 70, 77, 84 and 91 days after sowing in chickpea crop. Chlorophyll content was higher in healthy leaves as compared to diseased leaves yet there was reduction in chlorophyll content as the days advanced. The results revealed that mean value of chlorophyll-a (0.706 mg g^{-1} and 0.532 mg g^{-1}), chlorophyll-b (0.234 mg g^{-1} and 0.16 mg g^{-1}) and total chlorophyll (0.938 mg g^{-1} and 0.694 mg g^{-1}) in healthy and diseased leaves respectively and the mean chlorophyll content was (0.626 mg g^{-1} and 0.462 mg g^{-1}) in healthy and diseased leaves respectively.

The reduction in the chlorophyll content may be due to the production of alternariol mono methyl ether produced by *Alternaria tenuis* in tobacco that led to inhibition of chlorophyll production by the fungus (Pero and Main, 1970). The reduction of chlorophyll was mainly because of intensification of powdery mildew infection and defoliation due to the disease by Ashtaputre (2005).

5.1.2 Total phenol

Among all biochemical compounds, phenols are reported to play a determining role in providing the resistance or susceptibility of a host to parasitic infection. Many workers reported that higher content of phenolics in resistant genotypes than susceptible (Rubin and Aksenova, 1957; Raghunathan *et al*., 1958; Anahosur and Naik, 1985).

In the present investigation the total phenol changed with the days from 63 to 91 days. There was a gradual increase in total phenol content in both healthy and diseased leaves and found highest at 91 DAS with 3.87 mg g^{-1} and 3.08 mg g^{-1} in healthy and diseased leaves respectively. The total mean phenol content was 3.52 mg g^{-1} in healthy leaves as compared to diseased leaves 2.06 mg g^{-1}.

Post infectional increase in phenolics content could be due to their enhancement of synthesis, translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosides to yield free phenols or release from glycosidic esters by the enzymatic activity of host or pathogen (Noveroske *et al*., 1964) or due to migration of phenols from non-infected tissue (Farkas and Kiraly, 1962).

5.1.3 Total sugars

Sugars are precursors for the synthesis of phenolics, phytoalexins, lignin and callose. Hence, they play important role in defence mechanism of plant against invading pathogens. Generally high levels of total sugars, reducing sugars and non reducing sugars in the host plant are started to be responsible for disease resistance indicated by several workers (Bateman and Millar, 1966; Jayapal and Mahadevan, 1968).

In the current investigation of reducing sugar, non-reducing sugar and total sugar content were recorded in both healthy and infected leaves at 63, 70, 77, 84 and 91 DAS in chickpea crop. The results revealed that mean value of reducing sugar (2.24 mg g^{-1} and 1.78 mg g^{-1}), non-reducing sugar (1.61 mg g^{-1} and 1.42 mg g^{-1}) and total sugar (3.85 mg g^{-1} and 3.40 mg g^{-1}) in healthy and diseased leaves respectively. The total mean sugar content was 2.57 mg g^{-1} in healthy leaves as compared to diseased leaves 2.20 mg g^{-1}.

In general, these changes may be due to the infection by some pathogens which brings change in respiratory pathway and photosynthesis that are the vital process taking place inside the plant and leading to wide fluctuation in sugars (Farkas and Kiraly, 1962, Klement and Goodman, 1967 and Jayapal and Mahadevan, 1968). In susceptible varieties, disease development was more whereas, the mean sugar content comes down at later part of the crop growth. This indicated the utilization of these sugars by the invaded pathogens for their nutrition. Such nutritional utilization of sugars by the invading pathogens has been reported by Krog *et al.,* (1961).

5.1.4 Protein

In the present investigation it was observed that protein content was higher in healthy leaves than infected ones. In general, the protein content was decreased in both healthy and powdery mildew infected leaves from 63 DAS to 91 DAS. Lowest total protein was observed at 91 DAS with 4.60 mg g^{-1} and 3.53 mg g^{-1} in healthy and diseased leaves respectively. The total mean protein content was 6.33 mg g^{-1} in healthy leaves as compared to diseased leaves 5.04 mg g^{-1}.

It is well known fact that enzymes are proteins and the increased synthesis of proteins during the infection may be due to activation of enzymes which are essential for the synthesis of various defense chemicals. Macerating enzymes secreted by pathogens are involved in the pathogenicity of a wide range of plant pathogenic fungi (Peeran *et al.*, 2014).

5.1.5 Amino acids

An infection by pathogens brings about various changes in amino acids metabolism of the host plant. Amino acid functions as a nitrogen source for the pathogen and act as inhibitory to the activity of pathogen or precursors of various fungal toxic compounds, particularly phenolics.

In the present investigation it was observed that amino acid content was higher in healthy leaves than infected ones. In general, the amino acid content was decreased in both in healthy and powdery mildew infected leaves as the age of the crop advances. Lowest amino acid content was observed at 91 DAS with 0.60 mg g^{-1} and 0.30 mg g^{-1} in healthy and diseased leaves respectively. The total mean amino acid content was 1.12 mg g^{-1} in healthy leaves as compared to diseased leaves 0.74 mg g^{-1}.

It may be due to higher proteolytic enzyme activity in the susceptible genotypes due to pathogen or inhibition of protein synthesis in the host by (Mahatma *et al.*, 2009) or It may be due to proteolytic enzyme activity of the pathogen or inhibition of protein synthesis in the host (Mitter *et al.,* 1997).

5.2 Nutrient parameters of chickpea

5.2.1 Mineral

All living organisms require a continuous supply of large number of substances from outside to complete their life cycle, Similarly green plants have comparatively simple nutrient requirements and these are classified as macronutrients (N, P, K and Mg) and micronutrients (Fe, Mn, Cu, Zn, and B). They are essential for all the metabolic processes. In current investigation an attempt was made to know the effect of powdery mildew infection on mineral status in chick pea.

In response to infection by powdery mildew mineral status changes in chickpea powdery mildew, some elements comparatively increased *viz.,* Nitrogen, Potash, Magnesium, Zinc, Iron found highest in healthy leaves except Fe and B. Some elemental contents like Phosphorus and Molybdenum gradually increased in healthy and decreased in infected leaves. Copper content was found comparatively decreased.

The fluctuation in mineral content could be due to presence of powdery mildew pathogen, which utilizes the nutrients for its growth and development and due to the improper supply of nutrients to the infected area and subsequent death of the infected leaf. Reduction of nitrogen content may be due to utilization of free amino acids present in the pods by the pathogen (Hegde and Munjal, 1971). Powdery mildew infection shows potential increase in nutrient uptake of plant (Thite, 2013).

5.3 *In vivo* evaluation of chemicals

Use of fungicides to manage the disease is an age old practice in plant protection in the absence of resistant varieties for the disease and it is important to prevent the break down of resistance in commercial varieties. Hence, the efficacy of fungicide including new generation molecules will help in reducing the yield loss due to powdery mildew epidemics.

In the present investigation, a field experiment was conducted during *rabi* 2015-16 at ARS, Annigeri. Nine fungicides along with unprotected control were evaluated for their efficacy in disease control under natural epiphytotic conditions. The results after spray revealed that, among the nine fungicides myclobutanil (0.00 %) at 0.2 per cent and azoxystrobin (0.00 %) at 0.05 per cent reduced disease cent per cent and found significantly superior over other treatments, followed by difenconazole, tebuconazole, hexaconazole and propiconazole which were at par with each other and significantly superior over unsprayed treatment. Whereas, Wettable sulphur (8.89 %) at 0.3 per cent and taqat (8.15 %) at 0.2 per cent concentration were moderately effective and remained statistically at par with each other. Myclobutanil showed maximum BCR than azoxystrobin and also found to be effective in managing powdery mildew of chickpea.

Channaveeresh and Kulkarni (2015) screened twelve fungicides against *Erysiphe polygoni* causing powdery mildew in black gram, among all azoxystrobin found most effective.

Future line of work

Studies on biochemical, nutrient status and new molecules of fungicides for resistance in chickpea in the present investigation have opened up new areas of resistance exhibited by chickpea genotypes. Hence, the following future line of work is being suggested.

1) Histo-pathological studies on factors responsible for powdery mildew resistance exhibited by chickpea genotypes.
2) Many enzymes like peroxidase and poly phenol oxidase may be studied for clear understanding of biochemical nature operating in resistance against *Leveillula taurica* by chickpea genotypes.
3) Molecular markers responsible for the morphological and biochemical nature of the resistance genes need to be identified.
4) Spatial and temporal variability studies needs to be carried out by using Geographic information system (GIS), Global positioning system (GPS) and Remote sensing (RS).

6. SUMMARY AND CONCLUSION

The full potential of the chickpea is far from exploitation due to many biotic and abiotic stresses. Among the biotic factors, diseases contribute more for loss in the yield. These include powdery mildew caused by *Leveillula taurica*.

The present investigations include few important aspects *viz.,* morphological parameter was studied to understand the resistance in relation to chlorophyll and biochemical parameters like phenol, reducing sugars, total sugars, proteins and free amino acid and quantitative status of nutrients (N, P, K, Mg, Zn, Fe, Mn, Cu, and B) due to pathogen infection was conducted at Regional Agricultural Research Station, Vijayapur during *rabi* 2015-16. *In vivo* evaluation of fungicides against powdery mildew resistance in chick pea variety JG-11 was conducted during *rabi* 2015-16 at Agricultural Research Station, Annigeri. The results of the investigation are summarized here under.

As the days advanced from 63 to 91 DAS, the chlorophyll-a, chlorophyll-b and total chlorophyll contents were remarkably reduced and found to be higher in healthy leaves than infected ones. The mean total chlorophyll was 0.626 mg g^{-1} in healthy leaves as compared to diseased leaves 0.462 mg g^{-1}.

Similar trend was observed in biochemical parameters, where total mean phenol content was 3.52 mg g^{-1} as compared to diseased leaves 2.06 mg g^{-1} as the days advanced with 63 to 91 days there was a gradual increase in total phenol content in both healthy and diseased leaves. Sugars were recorded the total mean of 2.57 mg g^{-1} in healthy leaves as compared to diseased leaves 2.20 mg g^{-1}. As the days advanced with 63 to 91 days there was a gradual decrease in sugar content in both healthy and diseased leaves. Proteins recorded total mean of 6.33 mg g^{-1} in healthy leaves as compared to diseased leaves 5.04 mg g^{-1}. As the days advanced with 63 to 91 days there was a gradual decrease in protein content in both healthy and diseased leaves. Free amino acids recorded the total mean was 1.12 mg g^{-1} in healthy leaves as compared to diseased leaves 0.74 mg g^{-1}. As the days advanced with 63 to 91 days there was a gradual decrease in total phenol content in both healthy and diseased leaves.

With respect to nutrient analysis it is found a highly negative correlation between N, P, K, Mg, Zn, Mn, Cu and disease intensity, whereas a highly positive correlation was observed between Fe, B, and disease intensity. The healthy leaves had higher concentration of N, P, K, Mg, Zn, Mn and Cu and fewer amounts of Fe and B.

For the management of powdery mildew of chickpea new fungicide molecules were evaluated under natural epiphytotic conditions at ARS, Annigeri. Among nine chemicals tested myclobutanil @ 0.02 % and azoxystrobin @ 0.05 % found effective in managing the disease cent per cent and also recorded comparatively more yield. Myclobutanil showed maximum BCR than azoxystrobin and also found to be effective in managing powdery mildew of chickpea.

REFERENCES

Adinarayana, M., MahaLakshmi, S. M. and Koteswara, R. Y., 2012, Field Evaluation of Penconazole 10 EC (NS) against Powdery mildew in Urdbean. *J. Biopest.,* 5(2): 214-217.

Ahir, A. K., Brahmbhatt, A. B. and Patel, J. K., 2015, Field evaluation of fungicides against powdery mildew (*Erysiphe polygoni*) of green gram. *Pl. Dis. Res,.* 30 (2): 183-184.

Ajithkumar, K., Savitha, A. S., Biradar, S. A., Rajanna, B. and Ramesh, G., 2014, Management of powdery mildew and anthracnose diseases of chilli (*capsicum annuum* l.). *Pest Management in Hortic. Ecosystems.* 20(1): 80-83.

Akhileshwari, S. V., Amaresh, Y. S., Naik, M. K., Kantharaju, V., Shankergoud, I. and Ravi, M. V., 2012, Field evaluation of fungicides against powdery mildew of sunflower. *Karnataka J. Agric. Sci*., 25 (2) : 278-280.

Aly, A. A., Mahmoud, T. M., Mohamed, H. I. and Kamel. A. A., 2012, Examination of correlations between several biochemical components and powdery mildew resistance of flax cultivars. *Plant Pathol. J.,* 28(2): 149-155.

Anahosur, K. H. and Naik, S. T., 1985, Relationship of sugars and phenols of roots and stalk of sorghum with charcoal rot. *Indian Phytopath.*,38:131-134.

Anonymous, 2013, International chickpea and pigeon pea newsletter (ICPN), ICRISAT.

Anonymous, 2014, Directorate of economics and statistics, Department of Agriculture and Cooperation. Agricultural statistics- at glance 2014. pp. 94-96.

Ashry, A. N. and Mohamed, I. B., 2011, Impact of secondary metabolites and related enzymes in flax resistance and susceptibility to powdery mildew. *World J. of Agric. Sci.,* 7 (1): 78-85.

Ashtaputre, S. A., 2005, Studies on loss assessment, epidemiology and management of powdery mildew of chilli caused by *Leveillula taurica* (lev.) arn). *Ph.d. Thesis,* Univ. Agric. Sci., Dharwad, Karnataka (India).

Ashtaputre, S., Srikant Kulkarni, M. S. L. and Shivaprasad, M., 2007, Management of powdery mildew of chilli using triazoles. *J. Pl. Dis. Sci.,* 2(2): 132-134.

Attanayake, K. P. R. N., Glawe, D. A., McPhee, K. E., Dugan, F. M. and Chen, W., 2008, First report of powdery mildew of chickpea (*Cicer arietinum*) caused by *Leveillula taurica* in Washington State*. Pl. Health Prog*., 23(3): 23-28.

Audichya, P. and Thakare, B. B. L., 2000, Management of powdery mildew of opium poppy by systemic fungicides. *J. of Mycol. and Pl. Path.,* 30:103-104.

Bateman, D. F. and Millar, R. L., 1966, Pectic enzymes in tissue degradation. *Ann. Rev. Phytopath.,* 4: 119-146.

Bray, H. G. and Thorpe, W. Y., 1954, Analysis of phenolic compounds of interest in metabolism. In: *Meth. Biochem. Ann*., 1:27-52.

Channaveeresh, T. S. and Kulkarni, S., 2015, *In vitro* evaluation of fungicides, bioagents and botanicals against *Erysiphe polygoni* DC in black gram. *Inter. J. in Physical & Applied Sci.,* 2(3): 2394-5710.

Chavan, S. S. and Suryvanshi, A. P., 2014, Studies on biochemical changes in soybean infected with *Colletotrichum truncatum* (schw.). *Inter. Quarterly J. life Scie.,* 9(3): 1341-1344.

Dakshayani, R., Mummigatti, U. V., Srikant Kulkarni and Ravikumar, R. L., 2005, Screening green gram genotypes for powdery mildew using biochemical parameters. *Karnataka J. Agric. Sci.,* 18(2): 500-502.

Devi, P. A. and Prakasam, V., 2014, Efficacy of azoxystrobin 25 SC along with bioagents on chilli powdery mildew diseases under field condition. *World J. of Agric. Sci.,* 2(1): 008-012.

Dhanumjayarao, I. K., Jindal, P. C., Singh, R., Srivastava, G. C. and Sharma, R. C., 2006, Biochemical variability studies for disease resistance in grape germplasm against powdery mildew (*Uncinula negator*) (schw) bur along with some varietal characters. *Indian J. Agric. Res.,* 40 (3): 212 – 215.

Dhingra, M., Arora, N. and Aujla, I. S., 2013, Biochemical characteristics imparting resistance against *alternaria* blight in cauliflower genotypes. *Agric. Sci. Digest.,* 33 (2): 92 – 97.

Dhruj, I. U., Akbari, L. F., Khandar, R. R. and Vaishnav, M. U., 1996, Effectiveness of triazole fungicides against powdery mildew of fenugreek. *Pl. Dis. Res.,* 11(1): 86-88.

Farkas, G. L. and Kiraly, Z., 1962, Role of phenolic compounds in the physiology of plant diseases and disease resistance. *Phytopath. Zeitschrift,* 44: 105-150.

Gil, J., Nadal, S., Luna, D., Moreno, M. T. and De Haro, A., 1996, Variability of some physio-chemical characters in desi and kabuli chickpea types. *J. Sci. Food and Agric*., 71: 179-184.

Girija G., Chethana, B. S., Archana. S. R. and Bellishree, K., 2011, Comparative efficacy of myclobutanil, triadimefon and mancozeb against major fungal diseases of chilli. *Pest Management in Hortic. Ecosystems,* 17(1):42-47.

Gupta, S. H. and Shyam, K. R., 1998, Control of powdery mildew and rust of pea by fungicide. *Indian Phytopath.,* 51 (2) : 184-186.

Gurjar, P. S., Singh, S. K., Singh, A. K., Verma, M. K. and Beniwal, S., 2015, Screening and Biochemical Studies on Grape Genotypes for Powdery Mildew Infection under Sub-Tropical Conditions. *Indian J. Agric. Biochem.,* 28 (2) : 178-182.

Haware, M. P., 1998, Seed borne diseases of chickpea. Techn. Bull. No.1, ICRISAT, Hyderabad, pp. 32.

Hegde, R. K. and Munjal, R. L., 1971, Physiological changes in bean pods due to infection of *Colletotrichum lindemuthianum. Indian phytopath.,* 24: 186-188.

Hiscox, J. D. and Israelstan, G. E., 1979, A method for extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot*. 57: 132-134.

Ibrikci, H., Knewtson, S. J. B. and Grusak, M. A., 2003, Chickpea leaves as a vegetable green for humans: evaluation of mineral composition. *J. Sci. Food and Agric.*, 83: 945-950.

Jackson, M. L., 1973, Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi, pp. 111-205.

Jayapal, R. and Mahadevan, A., 1968, Biochemical changes in banana leaves in response to leaf spot pathogens. *Indian Phytopath*., 59: 963-967.

Jyosthna, M. K., Eswara Reddy, N. P., Chalam, T. V. and Reddy, G. L. K., 2004, Morphological and biochemical characterization of *Phaeoisariopsis personata* resistant and susceptible cultivars of groundnut (*Arachis hypogaea*). *Pl. Patho. Bulletin* 13: 243-250.

Klement, X. and Goodman, R. N., 1967, The hypersensitive reaction to infection by bacterial plant pathogens. *Ann. Rev. Phtopath.,* 05: 17-44.

Krog, N. E., Tourneau, D. L. and Hari, H., 1961, The sugar content of wheat leaves infected with stem rust. *Phytopath.,* 51: 75-77.

Ladizinsky, G., 1975. A new Cicer from Turkey. Notes of the Royal Botanic Garden Edinburgh 34: 201-202.

Lowry, O. H., Rosebrough, A. L. F. and Randal, R. J., 1951, Protein measurement with the Folin-phenol reagent. *J. Biol. Chem*., 193: 265-275.

Mahatma, M. K., Bhatnagar, R., Pinakin Dhandhukia and Thakkar, V. R., 2009, Variation in metabolites constituent in leaves of downy mildew resistant and susceptible genotypes of pearl millet. *Physiol. Mol. Biol. Plants*. 15(3)

Mandhare, V. K., Suryawanshi, A. V. and Jamadagani, B. M., 2005, Occurrence of powdery mildew (*Leveillula taurica*) on chickpea in Maharashtra. *J. Maharashtra Agric. Univ*., 30(3): 340.

Mishra, C. N., Kumar, S., Gupta, V., Tiwari, V. and Sharma, I., 2015, Utilization of chlorophyll content index (CCI) to infer yellow rust severity in wheat (*Triticum aestivum* L.). *J. of Applied and Natural Scie.,* 7 (1) : 38 – 42.

Mitter, N., Grewal, J. S. and Pal, M., 1997, Biochemical changes in chickpea genotypes resistant and susceptible to grey mould. *Indian Phytopath.,* 50 (4) : 490-498.

Moore and Stein, 1958, A modified nin-hydrin reagent for the photometric compounds. *J. Biol.Chem*., 211: 907-913.

Muehlbauer, F. J. and Singh, K. B., 1987, Genetics of chickpea (*Cicer arietinum* L.). In: M.C. Sexana and K.B. Singh (Eds:), The chickpea CAB International, Wallingford, Oxon, pp. 99-126.

Muhammad, S., Viswanatha, K. P., Chandrasekhara, K., Mudalagiriyappa, Nehru, S. D., Nagaraj, K. and Biradar, B. D., 2012, Powdery mildew on chickpea caused by *L. taurica*: A new report from south India. *Pulses Newsltr.,* 26(1): 4.

Nelson, N., 1944, A photometric adaptation of Somogyi method for determination of glucose. *J. Bio. Chem.,* 153: 375-378.

Nene, Y. L., Sheila, V. K. and Sharma, S. B., 1996, A world list of chickpea and pigeonpea pathogens. 5th Ed. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. p. 27.

Noveroske, R. L., Williams, E. B. and Kuc, J., 1964, 7-Glucosidase and polyphenol oxidase in appleleaves and their possible relation to *Venturia inaequallis*. *Phytopath.* 54: 98-103.

Panse, V. G. and Sukhatme, P. V., 1985, Statistical Methods for Agricultural Workers. ICAR Publications, New Delhi, India. pp. 359.

Parashar, A. and Lodha, P., 2011, Estimation of protein content in fennel (*Foeniculum vulgare*) infected with *ramularia* blight and powdery mildew. *Indian J. of Fundamental and Applied Life Sci.,* 2(1): 2231-6345.

Patil, L. C., Nadaf, H. L., Kalappanavar, I. K. and Megeri, S. N., 2011, Biochemical relationship in resistant and susceptible cultivars of spot blotch infected tetraploid wheat. *Karnataka J. Agric. Sci., 24 (4):* 520-522.

Peeran, M. F., Kuppusami, P. and Thiruvengadam, R., 2014. Pathogenesis of *Colletotrichum lindemuthianum* the incitant of anthracnose disease in beans mediated by macerating enzymes. *The Bioscan.* 9(1): 295-300.

Pero, R. and Main, C., 1970, Chlorosis of tobacco induced by Alternariol monomethyl ether produced by *Alternaria tenuis. Phytopath.,* 60: 1570-1573.

Raghunathan, R., Mahadevan, A. and Rangaswamy, G., 1958, A comparative study of the resistant and susceptible banana varieties to fungal diseases. *Phytopath.,* 49:141-152.

Philip, R. and Devadath, S., 1980, Studies on the physiology of bacterial blight infected tolerant and susceptible rice cultivars. *Phytopath.,* 101: 65-71.

Ramesh, Patil, M. B., Bheemaraya, Naik, M. K., Amaresh, Y. S. and Bheemanna, M., 2013, Evaluation of fungicides and bioagents for management of powdery mildew (*Leveillula taurica* (Lev.) Arn) of chilli in TBP and UKP- Command areas of Karnataka. *Env., and ecology.* 31 (1A): 381-384.

Reddy, M. N. and Sireesha, C. H., 2013, Role of oxidative enzymes and biochemical constituents in imparting resistance to groundnut (*Arachis hypogaea L.*) against stem rot disease caused by *Sclerotium rolfsii.* Bioresearch Bulletin, pp 36-41.

Rubin and Aksenova, V. A., 1957, Participation of the polyphenolase system in the defense reactions of potato against *Phytopthora infestans. Biochm.,* 22: 202-209.

Sankar, N. R. and Sreeramulu, A., 2009, Biochemical changes in teak leaves infected by powdery mildew fungus, *Unclnula tectonae* salm. *J.Pl.Dis.,* 4(1): 57-59.

Saxena, D. R. and Saxena, M., 2002, Optimal fungicidal spray to control powdery mildew of rainy season green gram. *Pl. Path. J.,* 18(2): 68-73.

Sankar. A. V. B. and Jindal, P. C., 2001, Biochemical resistance of grape genotypes against anthracnose. *Indian J. Agric. Res.,* 35(1): 44-47.

Sharmila, A. S., Kachapur, M. R. and Patil, M. S., 2004, Field evaluation of fungicides against powdery mildew (*Leveillula taurica* (Lev.) Arn) of chilli (*Capsicum annuum* L.). *J. Mycol. Pl. Path.,* 34(1): 98-99.

Shivanna, E., Arun Sataraddi, Janagoudar, B. S. and Patil, M. B., 2006, Efficacy of fungicides for the management of powdery mildew *Erysiphe cichoracearum* of okra. *Indian J. Pl. Prot.,* 34(1): 85-88.

Shukla, V., Singh, S. K. and Baghel, S., 2014, Role of biochemical constituents in imparting resistance to mungbean varieties against anthracnose [*Colletotrichum truncatum* (schw.) andrus and moore]. *International Quarterly J. of Env. Sci.,* Vol. VI: 141-145

Sindhan, G. S. and Parashar, R. D., 1981, Analysis of phenols, sugars and minerals in pea leaves in correlation with powdery mildew incidemce. Abstr. III. *International symp.* Plant pathology, IARI, New dehli, Dec. 11-14, pp.189.

Singh, K. P. and Pal, R., 2000, Evaluation of fungicides against powdery mildew of wheat. *Indian Phytopath.,* 53 (2) : 230-23.

Sunkad, G. and Kulkarni, S., 2006, Studies on structural and biochemical mechanism of resistance in groundnut to *Puccinia arachidis*. *Indian Phytopath.,* 59 (3) : 323-328.

Suresh, K. and Padaganur, G. M., 1990, Field evaluation of fungicides against powdery mildew of greengram. *J. of farm Scie.,*3: 3-4.

Suryawanshi, A. P., Wadge, A. G., Gawade, D. B., Kadam, T. S. and Pawar, A. K., 2009, Field evaluation of fungicides and botanicals against powdery mildew of munga bean. *Agic. Scie. Digest.,* 29(3): 209-211.

Sushmita, H., 2015, Epidemiological studies on chickpea powdery mildew caused by *Leveillula taurica* (lev.) Arnaud. *M. Sc. Thesis,* Univ. Agric. Sci., Dharwad, Karnataka (India).

Thite, S. V., Aparadh V. T. and Kore, B. A., 2013, Effect of powdery mildew infection on mineral status of *Dalbergia sissoo. Int. J. Res. in Pharmaceutical and Biomedical Sci.,* 4 (3), 841-846.

Upasana, R., Munshi, G. D. and Indu Sharma, 2005, Efficacy of fungitoxicants against powdery mildew of wheat (*E. graminis* f.sp. *tritici*). *Pl. Dis. Res.,* 20(2): 161-162.

Wheeler, B. E. J., 1969, An Introduction to Plant Diseases. John Wiley and Sons Ltd., London, p. 301.

Wheeler, H., 1975, Effects of *Helminthosporium victoriae* and victorin up on permeability. *Am. J. Bot.,* 50: 686-693.

BIOCHEMICAL AND NUTRIENT STATUS IN CHICKPEA DUE TO POWDERY MILDW (*Leveillula taurica* (Lev.) Arnaud)

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Abstract

Powdery mildew of chickpea caused by *Leveillula taurica* (Lev.) Arn. is a major menace for chickpea cultivation in recent years. The present investigation was conducted at Regional Agricultural Research Station, Vijayapur during *Rabi* 2015-16,

As the days advanced from 63 to 91 DAS, the mean total chlorophyll content in healthy leaves (0.626 mg g^{-1}) as compared to diseased leaves (0.462 mg g^{-1}) the chlorophyll-a, chlorophyll-b and total chlorophyll contents were remarkably reduced. Similarly total mean phenol content in healthy (3.52 mg g^{-1}) as compared to diseased (2.06 mg g^{-1}) and found gradual increase in phenol content in both healthy and diseased leaves. Total mean of sugar content in healthy leaves (2.57 mg g^{-1}) as compared to diseased leaves (2.20 mg g^{-1}). Total mean protein content in healthy leaves (6.33 mg g^{-1}) as compared to diseased leaves (5.04 mg g^{-1}). Total mean of free amino acids in healthy leaves (1.12 mg g^{-1}) as compared to diseased leaves (0.74 mg g^{-1}) and these found gradual decrease in both healthy and diseased leaves.

With respect to nutrient analysis it is found a highly negative correlation between N, P, K, Mg, Zn, Mn, Cu and disease intensity, whereas a highly positive correlation was observed between Fe, B, and disease intensity. The healthy leaves had higher concentration of N, P, K, Mg, Zn, Mn and Cu and fewer amounts of Fe and B.

For the management of powdery mildew of chickpea new fungicide molecules were evaluated under natural epiphytotic conditions at ARS, Annigeri. Among nine chemicals tested myclobutanil @ 0.02 % and azoxystrobin @ 0.05 % found effective in managing the disease cent per cent and also recorded comparatively more yield. Myclobutanil showed maximum BCR than azoxystrobin and also found to be effective in managing powdery mildew of chickpea.