

Recent Research in Agriculture and Plant Sciences



Editors

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Preface

This Edited book “Recent Research in Agriculture and Plant Sciences” is based on the proceedings of the “2nd Online International Conference on Advance Interdisciplinary Research (ICAIR-2023)”, Jointly Organized by Digvijay Nath Post Graduate College, Gorakhpur, U.P. & Science-Tech Institute run by Manraj Kuwar Singh Educational Society, Lucknow, U.P. during April 07 -09, 2023. The aim of this conference was to bring together the young as well as experienced researchers on one platform to discuss the recent findings in the aforesaid areas of Physical and Chemical Sciences. After peer reviewing process, relevant research papers were finally included in this book as chapters.

The present volume is based on the contributions made by various authors on different important topic of “**Recent Research in Agriculture and Plant Sciences**” and introduces the subject along the following topics: Plant diseases-A major limiting factor in pulse production; Natural Resource Management; Weed Biology and Ecology; Development of Hybrid Time Series Models for Forecasting Total Rice Production in Assam; Common fungal diseases of Millet from all over the world; Awareness and Impact of Air Pollution on Biodiversity: Survey an Approach to Find Solution; Screening of some antipathogenic botanicals in vitro and field conditions against *Colletotrichum falcatum* causing red rot of sugarcane; *Rigidoporus vinctus*, an endophytic fungus with antioxidant and antimicrobial properties, from the shoot of *Bambusa tulda*; Investigation of bioactive molecules of *R. communis* by Gas Chromatography-Mass Spectrometry; and Phytochemical analysis and evaluation of antioxidant activity in methanolic extracts of *Macrotyloma uniflorum* (Lam).

We must place on record our sincere gratitude to the authors not only for their effort in preparing the papers for the present volume, but also their patience in waiting to see their work in print. Finally, we are also thankful to our publishers **Mrs. Shweta Singh** M/S MKSES Publishers, Lucknow for taking all the efforts in bringing out this volume in short span time.

Editors

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Chapter: 1
Plant diseases-A major limiting factor in pulse production

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Abstract: Agriculture is the backbone of the Indian economy. Pulses are one of the important groups of agricultural crops in India. They are rich source of plant-based protein therefore they are an essential component of Indian diet specially in the vegetarian society. They are naturally low in fat, full of nutrients and have high amounts of dietary fiber. Pulses are important for sustainable agriculture due to its capability of biological nitrogen fixation in the soil. They provide nutritional security to weaker sections of the society. They reduce greenhouse gas emissions and hence, become more ecological to agriculture and food industry. Pulses are grown all over the world. India is bestowed with a large number of pulse crops grown throughout the year in different cropping systems. Mung bean and chickpea are two important pulse crops in Asia which are rich in nutrients and considered as food as well as cash crop. These crops are suffered with significant economic losses annually because of numerous biotic and abiotic factors. There are a number of diseases which are caused by fungi, bacteria and viruses which is the major limiting factor in the production of pulses and is responsible for huge losses in its production. This chapter reviews the importance of pulses particularly with regard to mung bean and chickpea, and the important diseases hampering their production.

Introduction

The economic transformation of India which is a developing country, crucially depends on performance of its agriculture as well as its allied sector. This sector plays a major role in the rural livelihood, employment as well as food security of the nation. The proportion of population of India which depends directly or indirectly on agriculture for employment is more than any other sector because approx. 70% of the rural household still depends primarily on agriculture for their livelihood. Agriculture not only provide food to the entire population but it also provides raw materials to the industries, as well as feed and fodder to the livestock. The greatest challenge to the agriculture in the coming years is to provide sufficient food to the rapidly increasing population in order to combat with hunger and malnutrition.

Importance of pulses

Pulses are an essential accompaniment in the diet along with staple food like wheat and rice. They play an essential role in improving the livelihood as well as nutritional security especially in the underdeveloped and developing countries (Gepts *et al.*, 2005; Cannon *et al.*, 2009). India is largest producer and consumer of pulses in the world sharing 26% of the world production and 30% of the world consumption (Tiwari and Shivhare, 2017). In India, pulses account for around 20% of total area under cultivation of food grains and it contributes approx. 7-10% of the production of total food grains. The main pulse growing regions with high productivity are Madhya Pradesh, Maharashtra, Andhra Pradesh, Rajasthan, Uttar Pradesh, Karnataka, Tamil Nadu, and other states (Fig. 1). The percent share of pulse production is increasing day by day (Fig. 2).

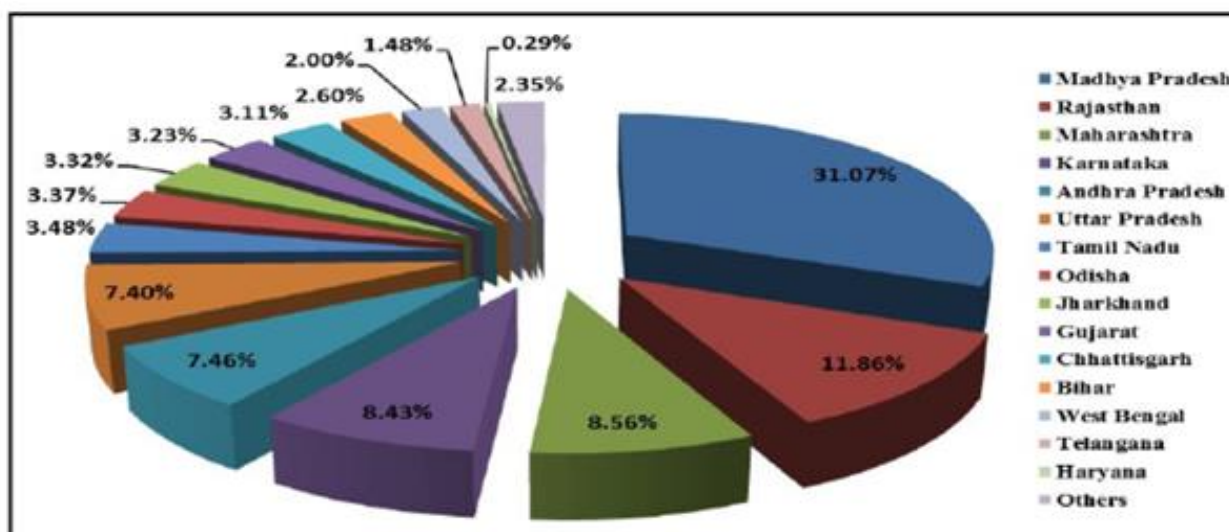


Figure 1: Share of Indian states in total production of pulses. (Anonymous, 2016)

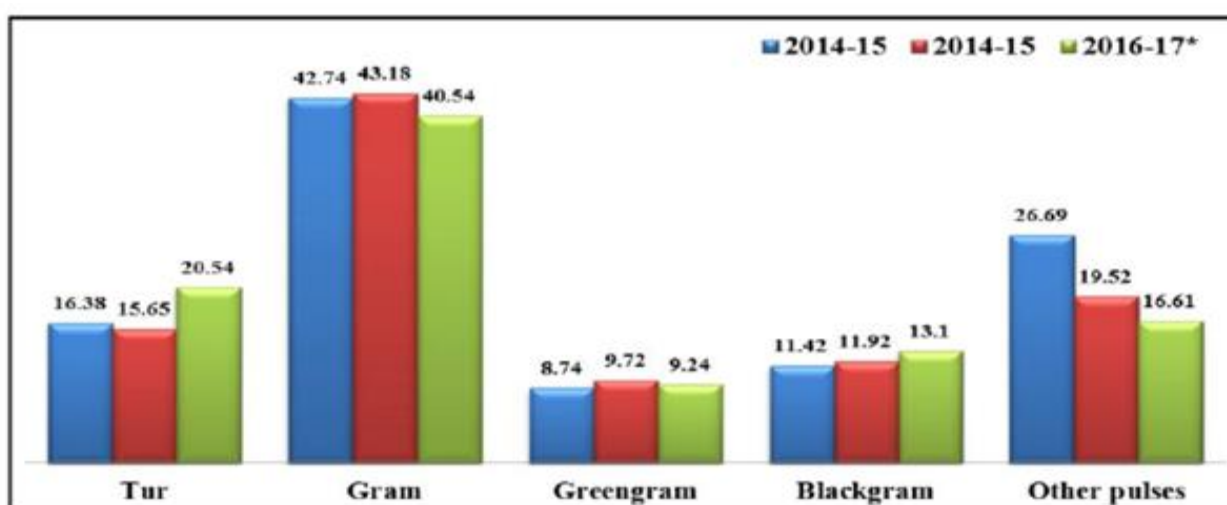


Figure 2: Share of different pulses in total production over the year (%)
(Source: Agricultural Statistics at a Glance, 2016 [*Based on 3rd Advance Estimates for 2016-17])

In India, pulses have been considered as poor man's only source of protein. Since, pulses are the main source of protein for the vegetarian populations hence, are important for their food security (Young, 2003; Weeden, 2007). About 15% of the dietary protein is met from the pulses in India as compared to 7% in the world.

Pulses are rich source of vitamins, minerals and essential amino acids. Pulses contain high content of proteins as well as carbohydrates and low content of fat (Tharanathan and Mahadevamma, 2003). They are the cheapest as well as easily accessible source of the required proteins (Broughton *et al.*, 2003). They are also relatively rich in two essential micronutrients, namely calcium and iron (Grewal *et al.*, 1988). Humans require proper amount of proteins as well as essential amino acids that are important for their proper body functioning. As pulses contains essential nutrients, they help the body to fight for mineral and vitamin deficiencies and diseases. It has been recorded that about 92% of the pulses are grown in rainfed areas. Erratic and uncertain behaviour of the monsoon disturb the timely sowing of these crops, which are normally chosen for the marginal and submarginal lands (Upadhyay *et al.*, 1998).

Pulses are legumes which belong to the family leguminosae and are endowed with unique quality of maintaining and restoring soil fertility through symbiotic relationship with root nodule forming bacteria as well as conserving and improving physiochemical properties of the soil by virtue of their deep root system (Pal, 1996; Biederbeck *et al.*, 2005; Zhu *et al.*, 2005).

Legumes have diverse uses. Some of them are used as green manure or fodder, and also used in vegetable oil extraction especially soybean and groundnut (Ofua and Akhidue, 2005). They also play an important role in alleviating greenhouse gases (Lemke *et al.*, 2005). Pulses are used alternatively in each cropping pattern that is either conventional or commercial production system (Siddique *et al.*, 2012). In this chapter, the significance and important diseases of mung bean and chickpea will be discussed.

Mung bean and chickpea

Mung bean (*Vigna radiata* L.) is an important food legume along with cash crop in Asia. The crop is a chief source of high quality protein which is low in cost as compared to other sources of protein (Table 1). It makes around 14% of the total protein of average diet of an Indian. Mung bean consists of approx. 25-28% protein, 3.5-4.5% fiber, 62-65% carbohydrates, 4.5-5.5% ash and 1.0-1.5% oil. Mung bean is rich source of vitamin A, B, C, niacin as well as important minerals such as potassium, calcium, and phosphorus that plays a great role for maintaining the functions of

human body (Gopalan *et al.*, 1995; Itoh *et al.*, 2006). Mung bean is consumed either as the whole seed or in the split form. It is also used as flour, or sprouts, hence, it makes an important source of protein (Ramakrishnan *et al.*, 2019). It forms a balanced diet when it is taken with cereals. Besides as an important food stuff, this legume improves fertility of soil by fixing the nitrogen through symbiosis with root-nodule forming bacteria (SubbaRao, 1972). Mung bean fits well in various multiple and intercropping systems as it is short duration in nature.

Table 1: Nutritive value of Mung bean (Source: Drlogy, 2023)

Mung bean (Nutrition profile)		
Serving size 100g		% Daily values
Calories	30 kcal	2%
Protein	3 g	6%
Total Carbohydrate	5.9 g	2%
Sugars	4.1 g	
Dietary fiber	1.8 g	7%
Total Fat	0.2 g	0%
Vitamin C	13.2 mg	22%
Vitamin K	33 µg	41%
Riboflavin	0.1 mg	7%
Thiamin	0.1 mg	6%
Iron	0.9 mg	5%
Folate	61 µg	15%
Phosphorus	54 mg	5%
Magnesium	21 mg	5%
Manganese	0.2 mg	9%
Copper	0.2 mg	8%
% Daily values are based on a 2000 calories diet.		

Chickpea (*Cicer arietinum* L.) commonly called as ‘Gram’ is another and one of the vital pulse crop in the world (Singh, 1988; Singh, 1990; Ali *et al.*, 2003; Pande *et al.*, 2006), originated from West Asia with an annual production of approx. 5.47 million tons and shares around 65% of the total production (FAO, 2007). The crop is under cultivation of 13,111,816.6 ha of area with a production of 969.5 kg ha⁻¹ and 63,669,396 million tons over the world from 2013 to 2017 (FAOSTAT, 2019). The chickpeas are of mainly two types i.e. Desi and Kabuli and they are grown in 50 countries with around 90% of its production as well as consumption in the developing countries (Joshi *et al.*, 2001). Being a winter season or Rabi crop, it is primarily cultivated as a

rainfed crop on the residual moisture conserved from the preceding monsoon. The crop is of great significance in India and provides good nutritive value (Table 2).

Table 2: Nutritive value of Chickpea (Source: Drlogy, 2023)

Chickpea (Nutrition profile)		
Serving size 100g		% Daily values
Calories	164 kcal	8%
Protein	8.9 g	18%
Total Carbohydrate	27.4 g	9%
Sugars	4.8 g	
Dietary fiber	7.6 g	30%
Total Fat	2.6 g	4%
Vitamin C	1.3 mg	2%
Vitamin A	27 IU	1%
Vitamin B6	0.1 mg	7%
Riboflavin	0.1 mg	4%
Thiamin	0.1 mg	8%
Iron	2.9 mg	16%
Pantothenic acid	0.3 mg	3%
Choline	42.8 mg	
Folate	172 µg	43%
Calcium	49 mg	5%
Phosphorus	54 mg	5%
Magnesium	48 mg	12%
Potassium	291 mg	8%
Manganese	1 mg	9%
Copper	0.4 mg	18%
Selenium	3.7 µg	10%
Zinc	1.5 g	10%
% Daily values are based on a 2000 calories diet.		

Besides being an excellent source of protein, chickpea is also rich in carbohydrates, minerals such as magnesium, calcium, potassium, zinc, phosphorus, iron in addition to vitamins like thiamine and niacin (Williams and Singh, 1987). Besides being a significant human food item plus animal feed, chickpea also have a key role in sustaining the fertility of soil through fixation of nitrogen (N_2) up to 141 kg nitrogen per hectare (Rupela, 1987). However, the amount of N_2 fixation by chickpea is greatly influenced by the crop management practices (Ali *et al.*, 1998). The diseases

affects all plant parts including the stems, leaves and pods, leading to development of lesions as well as breaking of the shoot (Pande *et al.*, 2005).

Plant diseases affecting pulse production

The average/capita consumption of the pulses in India is 37 grams which is much lower than that the WHO has recommended i.e. 46 gram pulse grains/capita/day to the people of underdeveloped and developing countries (Pal, 1996). Shortage of protein is one of the major issues worldwide because around one billion people are facing the deficiency of protein and nutrients and therefore, they suffer from malnutrition (Godfray *et al.*, 2010). Majority of Indian population subsists on vegetarian diet and more than 250 million people live below poverty line, hence for them pulses is the only source of protein. Therefore, priority should be given on sufficient quantitative and environmentally safe production of pulses at affordable price.

The low productivity of mung bean and chickpea is as a result of biotic as well as abiotic constraints, poor management practices as well as unavailability of better quality seeds of the improved varieties to the farmers (Chauhan *et al.*, 2010). Pulses specially mung bean and chickpea suffer losses as a result of diseases, insects, drought, salinity, waterlogging as well as a variety of other stress factors (Bailey-Serres and Voisenek, 2008; Singh and Singh, 2011; Hanumantha Rao *et al.*, 2016). In addition to abiotic stresses, diseases and insect pests are responsible for the decline and uncertainty in pulse production (Grewal, 1983; Grewal *et al.*, 1988; Jadon *et al.*, 2020).

Diseases of mung bean

Mung bean is affected by a number of diseases, out of which the important one are yellow mosaic, dry root-rot, powdery mildew, anthracnose, tan spot, cercospora leaf spot (CLS), and halo blight (Singh *et al.*, 2000; Shahid and Khan, 2016 a,b; Pandey *et al.*, 2018). The major fungal diseases of mung bean are dry root rot caused by the fungus *Macrophomina phaseolina*, CLS caused by the pathogen *Cercospora canescens*, anthracnose caused by the fungus, *Colletotrichum acutatum*/*C. truncatum*/*C. gloeosporioides* and powdery mildew (PM) caused by *Podosphaera fusca*/*Erysiphe polygoni*). The other fungal diseases which are comparatively less important are *Alternaria* leaf spot caused by *Alternaria alternate*, web blight caused by *Rhizoctonia solani* and, Fusarium wilt caused by *Fusarium oxysporum* and *F. solani* (Ryley and Tatnell, 2011; Pandey *et al.*, 2018). Mung bean yellow mosaic disease which is also called as MYMD is a major viral disease of mung bean (Singh *et al.*, 2000; Noble *et al.*, 2019), which is caused by a number of begomoviruses and

transmitted by an insect whitefly named *Bemisia tabaci* (Nair *et al.*, 2017). The important bacterial diseases of mung bean include bacterial leaf spot (BLS) due to *Xanthomonas campestris* pv. *phaseoli*, tan spot caused by *Curtobacterium flaccumfacien* pv. *flaccumfaciens* and Halo blight caused by *Pseudomonas syringae* pv. *phaseolicola*.

The diseases pose a serious risk to the cultivation of mung bean crop (Shahid and Khan, 2019). The economic losses caused by MYMD in India account for up to 85% yield losses (Karthikeyan *et al.*, 2014). Reports of reduction in the yield of mung bean because of dry root rot were estimated 10-44% from India as well as Pakistan (Kaushik and Chand, 1987; Bashir and Malik, 1988). Losses in yield reduction by CLS in Pakistan and different states of India were reported to be 97% (Iqbal *et al.*, 1995; Chand *et al.*, 2012; Bhat *et al.*, 2014). Yield losses of 30-70% by anthracnose (Kulkarni, 2009; Shukla *et al.*, 2014), 33-44% by Rhizoctonia root-rot (Singh *et al.*, 2013) and 40% by powdery mildew (Khajudparn *et al.*, 2007) in India were reported. Whereas, the minor fungal diseases were reported to cause 20% yield loss because of Fusarium wilt disease (Anderson, 1985) and 10% because of Alternaria leaf spot disease (Maheshwari and Krishna, 2013).

Diseases of chickpea

Chickpea is susceptible to numerous pathogens and pests. Pathogens which affect chickpea crop includes fungi, bacteria, viruses and nematodes that lead to serious global economic losses (Nene and Reddy, 1987; Haware, 1998; Singh and Sharma, 1998; Singh *et al.*, 1999; Manjunatha *et al.*, 2011; Pande *et al.*, 2012). Among them wilt, blight, root rots, grey mould, etc. are very important diseases and causes economic losses to chickpea in fields. Total yield losses due to consequence of plant diseases may reach up to 25% in the western countries and 50% in the developing countries. Out of this, one-third losses occurred because of fungal infection (Bowyer, 1999; Wazir, 2019). The fungal diseases affect the vigor and reduce the yield which varies with the plant age, soil type and environmental conditions. Ascochyta blight due to *Ascochyta rabiei* is the one of most disastrous disease among all the fungal diseases of chickpea, causing a significant decline in the yield and quality of seeds. (Asrat, 2015; Megersa *et al.*, 2017; Addisu *et al.*, 2023; Foresto *et al.*, 2023). Amin and Melkamu (2014) have reported yield losses up to 41% due to epidemics caused by Ascochyta blight. *Fusarium* wilt is considered a very dangerous disease and causes significant losses to chickpea, pea, lentil etc. worldwide (Infantino *et al.*, 2006; Chen *et al.*, 2011; Taheri *et al.*, 2017, Chatterton *et al.*, 2019). Its causal organism, *Fusarium oxysporum* hinder

emergence, causes plant wilting, produce severe rot symptoms on chickpea roots and causes heavy losses to the crop (Safarieskandari *et al.*, 2021; Belay *et al.*, 2022).

Dry root-rot caused by the fungus *Macrophomina phaseolina* is also a very important disease of chickpea (Andrews and Hodge, 2010; Foyer *et al.*, 2016; Rai *et al.*, 2022). Due to this disease, yield losses as high as 100% have been reported in the susceptible varieties under the favorable conditions for the disease (Gupta and Sharma, 2015). Besides dry root-rot, there are other root-rots in this crop for e.g. wet root-rot due to *Thanatephorus cucumeris*, previously called as *Rhizoctonia solani*, collar rot due to *Athelia rolfsii* and black root-rot due to *Fusarium solani* (Ghosh *et al.*, 2013; Kaur *et al.*, 2012; Sharma *et al.*, 2015). Botrytis grey mould whose causal organism is *Botrytis cinerea* is an important destructive disease of chickpea (Davidson *et al.*, 2004; Pande *et al.*, 2004; Pande *et al.*, 2005; Khan *et al.*, 2010, 2011; Moparthi *et al.*, 2023). It is an economically devastating fungal disease, and this fungus, *B. cinerea* also aids as a model species for the interactions between the plant and the necrotrophs (Fillinger and Elad, 2016). Stem rot of chickpea due to *Sclerotinia sclerotiorum* and *S. trifoliorum* is also an important disease of chickpea (Njambere *et al.*, 2008). *Sclerotinia* is a necrotrophic, host generalist fungus which can cause significant economic losses in the chickpea yield (Leite *et al.*, 2016; Webster *et al.*, 2020; Mwape *et al.*, 2021). Up to 100% crop yield losses have been reported due to this disease in a conducive environment (Fuhlbohmer *et al.*, 2003). Another disease known as rust caused by the fungus *Uromyces ciceris-arietini*, which is a foliar disease of chickpea, have been reported to cause severe damage to chickpea and its infection led to premature defoliation and later on, likely plant death (Nargund *et al.*, 2011; Patil, 2013; Sunilkumar Shirasangi, *et al.*, 2017; Kumbar, 2021). Other diseases of chickpea e.g. Alternaria blight caused by *Alternaria alternate*, Colletotrichum blight by *Colletotrichum dematium*, Phoma by *Phoma medicaginis*, Stemphylium blight by *Stemphylium sarciniforme*, Powdery mildew by *Leveillula taurica*, Verticillium wilt by *Verticillium albo-atrum*, Collar rot by *Sclerotium rolfsii*, Phytophthora root rot by *Phytophthora medicaginis*, Pythium root and seed rot by *Pythium ultimum*. Some bacterial diseases also affect chickpea including bacterial blight due to *Xanthomonas campestris* pv. *cassia*, bacterial leaf spot due to *Burkholderia andropogonis*. Important viral diseases of chickpea are Stunt viral disease by leaf roll virus and Mosaic viral disease by alfalfa mosaic virus.

Plant disease management

Plant disease management is necessary to prevent productivity losses of economically important crops due to plant pathogens (Shahid *et al.*, 2023) Plant disease management is based on some important basic principles which are as follows.

Avoidance and Exclusion

Exclusion of plant diseases includes those practices which are designed to keep pathogens, vectors as well as infected plants away from disease-free areas. This method involves cultural practices that help to avoid the inoculum from entering into the field.

Eradication

Eradication comprises of destroying, eliminating or inactivating a disease causing organism after its establishment in a particular area.

Protection

Protection means to establish a physical or chemical barrier in between the host and disease causing agent.

Resistance

This method includes planting of resistant varieties. Varieties that are resistant to various diseases can be grown which prevent huge crop losses (Khan *et al.*, 2012 a).

Therapy

Therapy is that method of plant disease management which is used after infection has already occurred. This is achieved by incorporating a chemical control agent into the plant to inhibit the progress of disease development. It includes use of fungicides to control the disease after it has already spread in an area.

Conclusion

Plant diseases are major bottlenecks in realizing the potential yield of agricultural crops including mung bean and chickpea. In order to prevent the losses due to plant pathogens, strategies/approaches are required for effective management of plant diseases. Disease management approaches can be categorized into chemical, physical, as well as biological approaches (Khan *et al.*, 2012 b). Generally, a combination of physical and chemical methods are very much useful in the management of yield losses in crop due to plant pathogens, but their high

cost, hazardous effects on the human health along with the environment, accumulation of agrochemicals in the food, secondary pest infestation, and resistance development in pests do not promote its application in the agroecosystem. The chemical fungicides pose deleterious impact on beneficial microorganisms that inhabit in the soil and confers host susceptibility to the pathogens (Heydari and Pessarakli, 2010). On the other hand, strategies which are based on the target specific action of biological control agents, the metabolic products of the biological control agents and the plant products against the potential plant pathogens are much striking as well as promising from the environment point of view. However, the application of biological control agents and phytochemicals under field condition is still a major challenge for the management of the crop productivity losses due to numerous plant pathogens.

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Chapter: 2
Natural Resource Management
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❖ Natural Resource management

Promising technologies in natural resource management are related to improving water use efficiency, increasing input use, especially of fertilizer, and conserving soil and water resources.

(A) Water use

Water is an essential input for agriculture and allied activities. India accounts for 4% of world's water share and 17% of the world population. The -irrigated area has reached to more than 40% through ground, surface, and -other sources. Despite abundant availability of water, the World Resource -Institute categorized India into the high-water stress category. It is mainly due to the fact that the ground water levels' significantly falling in India and its condition is highly alarming in states such as Punjab and Haryana (Singh *et al.* 2020). In such a situation, conserving ground water and improving water use efficiency are an utmost priority for the sustainability of agriculture. Roughly, two-third of the cultivable land in India is rainfed while the remaining 39 -million ha land is irrigated by groundwater and 22 million ha by canals (Dhawan 2017). Adoption of water-efficient technologies is one of the most prominent pathways to address the sustainability of agriculture. This section provides a brief review of key technologies to better understand how these impacted the sustainability of agriculture and farmers' income. The assessment includes micro--irrigation technologies, land-leveling technology, agronomic practices and multiple water use systems to understand the adoption barriers and the potential impact.

Adoption of micro-irrigation technologies such as sprinkler and drip irrigation have a huge potential to improve water use efficiency and increase agricultural productivity (Namara *et al.* 2005; Verma and Namara 2006; Kumar 2016; Bahinipati and Viswanathan 2019). These studies show a significant rate of growth in the adoption patterns of micro-irrigation technologies overtime.

Top 5 states are Andhra Pradesh (51% of the gross irrigated area), Karnataka (49%), Maharashtra (34%), Tamil Nadu (29%) and Gujarat (22%). Strikingly, the area covered under micro irrigation in Punjab is merely 1% despite the fast depleting ground water table. In contrast, the neighbouring state Haryana has 10% area under micro-irrigation, especially in areas where groundwater quality is brackish. The low adoption of micro-irrigation technologies in Punjab and Haryana is mainly due to the dominance of rice cultivation. However, it may be pointed out that research is still in progress as to how to use micro-irrigation in rice crop. In terms of impact, the study by Bahinipati and Viswanathan (2016) found that more than 80% of the surveyed farmers reported improvement in water and agricultural productivity. Interestingly, more than 60% of them reported savings in labour and energy use. A study by Namara *et al.* (2005) reported a very high elasticity of the yield in response to the application of micro-irrigation technologies. In the selected crops (banana, groundnut and cotton), the application of micro-irrigation technologies, compared to traditional methods of irrigation, resulted in a significant yield improvement in terms of elasticity's in the range of 0.51 to 1.21. Despite saving irrigation water and increasing agricultural productivity, the adoption of micro-irrigation has been very slow. The main reason for the slow rate of adoption of micro-irrigation systems is the high cost of establishing the system. There are suggestions that low-cost of micro-irrigation systems is the precondition for their large scale adoption. As pointed out by Kumar (2006), there are other constraints, as listed below:

- (i) Unreliable access to ground water.
- (ii) Less independence across farmers in extracting water.
- (iii) Mismatch between micro-irrigation system and existing cropping patterns.
- (iv) High opportunity costs of pumping ground water.
- (v) Poor knowledge due to weak extension services.
- (vi) Fragmented and small size of land holdings.

Subsidies in micro-irrigation systems also play an important role in their adoption. To promote micro-irrigation system, the Government of Gujarat has made the following provisions adoption of micro-irrigation technologies is compulsory for getting new electricity connection and an additional 10% subsidy is extended for the adoption of micro-irrigation technologies. These provisions have increased the adoption of sprinkler and drip -irrigation systems (Bahinipati and Viswanathan 2016). Land levelling is another important intervention to improve input use efficiency, especially of water. Several efforts have been made in the

past to promote laser land levelling (LLL), especially in north-west states of India. These efforts have shown a positive and significant impact in (a) adopting micro-irrigation technologies, (b) improving water use efficiencies, and (c) increasing agricultural productivity (Jat *et al.* 2006; Bhatt and Sharma 2009; Sapkal *et al.* 2019). The projections reveal that adoption of LLL in 2 million ha area would conserve 1.5 million hectare-meter of irrigation water and save 200 million -litres of diesel (Jat *et al.* 2006). Adoption of LLL also increases cultivated area due to reduction in bunds and channels. The gains of LLL are as high as Rs. 20,000 per ha (Sapkal *et al.* 2019). Main adoption barriers for LLL are:

- (a) Small size of land holdings
- (b) High service charges for LLL
- (c) Scarcity of technical manpower and adequate skills
- (d) Lack of adequate knowledge. Further

There are reports that information about technology through farmer-to-farmer communication and private traders, and participation in agricultural training and membership in local agricultural institutions increase the likelihood and the intensity of adoption of LLL (Aryal *et al.* 2018). The LLL is characterized as the precursor technology for resource conservation (Jat *et al.* 2006).

(B) Inorganic fertilizer

Application of inorganic fertilizers and pesticides as per the recommended quantities has contributed to the increase in agricultural productivity and farmers' income. Their application, in combination with improved cultivars and irrigation, has significantly increased agricultural production. However, there is a wide disparity across states and farm size in the application of fertilizers and pesticides.

The better off states and large farmers are using excessive fertilizers and pesticides, while rainfed areas and small and marginal farmers are using less than the recommended doses. There are three challenges in optimum use of fertilizers and pesticides:

- (i) Affordability of fertilizers and pesticides
- (ii) Balance and judicious use of fertilizers and pesticides
- (iii) Environmental degradation as a result of their injudicious and indiscriminate use.

To address affordability, the Government of India provides a large amount of subsidy to make fertilizers affordable it amounted to about Rs. 80,000 crore in 2020-21. According to the government portal, the number of farmers who purchased the fertilizers are about 10.5 crore (out of 14.5 crore farmers), which suggests that only 70% of farmers are applying fertilizers and pesticides for cultivation activities. More detailed studies are to be conducted to better understand the key drivers of not using fertilizers by the remaining 30% of the farmers. Is it because of inaccessibility and or affordability? It would be useful to characterize these farmers and develop their typology so that appropriate policy and institutional arrangements can be made.

A large number of farmers in better endowed regions are overusing these inputs. To address the inappropriate use of fertilizers, the Government of India in 2014 initiated a 'Soil Health Card Scheme' so that farmers could apply soil test based nutrient fertilizer to maximize their profit. The optimum use of nutrient management has implications on input costs, productivity and -profitability (Joshi *et al.* 2019). Role of micro-nutrients, especially Zn and Mn, is also important. A programme, known as Bhoochetna was launched by the Government of Karnataka to promote micro-nutrient. The programme was aimed to improve the soil quality and to promote the balanced use of macro- and micro-nutrients. The programme yielded a positive impact on agricultural productivity (Joshi *et al.* 2019). Moreover, the Government of India advised the farming community to reduce fertilizers use and encouraged more zero-budget natural farming and the organic manure for the agricultural sustainability. However, its scientific validity is yet to be confirmed.

Disproportionate use of fertilizers and less application of organic manures has led to deterioration of soil health (Chand and Pandey 2008). Over the years, continuous application of excessive quantity of inorganic fertilizers has been adversely affecting the agricultural productivity and sustainability of agriculture (Baweja *et al.* 2019; Srivastava 2020). The adverse effects of excessive and injudicious use of fertilizers have resulted in the deterioration of soil health, loss of micro-flora and other organisms, and deterioration in the quality of groundwater. As noted earlier, the partial factor productivity of fertilizers has significantly declined during the last three and a half decades. On-farm experiments further reveal that the current fertilizer management patterns are depleting carbon and micronutrient availability, and thereby, adversely affecting agricultural production and income, and ruining soil health and water quality. Smallholders are more vulnerable to such adverse effects due to disproportionate fertilizer use. Higher subsidies on nitrogenous fertilizer induce greater urea

use compared to phosphorous, potash and other micronutrients (like manganese, zinc and boron). The fertilizer subsidy is also thinly distributed to smallholders due to their large number. On a per holding basis, smallholder farmers get about 14 times less fertilizer subsidy than what large farmers get (Government of India 2016). In 2015, entire urea is sold with neem coating. The neem coated urea has multiple benefits of saving urea, improving nitrogen use efficiency and increasing crop yields. Besides, it minimizes the leakages of fertilizer from agriculture to the non-agriculture sector, and thereby, reduces the subsidy burden on fertilizer. More research is needed to assess the adoption and impact of neem coated urea on all aspects of agriculture.

(C) Watershed

Watershed technology is considered to be a boon for the rainfed areas. It involves conservation and judicious use of rainwater for increasing agricultural production and controlling soil erosion. Watershed programme in India was designed based on the -research efforts made at eight locations by the Indian Council of Agricultural Research (ICAR). The programme involves connecting technologies with institutional arrangements, financial provisions, and capacity development for greater people's participation and effective governance for management of watersheds. The programme was designed for rainfed areas with multiple purposes, namely, increasing farmers' income, controlling soil erosion and conserving rain water.

Watershed programme in rainfed areas serves as an important tool for water resources development, groundwater recharge and socio-economic improvement (Joshi *et al.* 2008). Some of the structures and agronomic practices of watershed programme -include raised-bed planting, ridge-furrow method of sowing, sub-surface irrigation and precision farming all of these provide a great potential for improving water use efficiency (Gregory 2004; Singh *et al.* 2014; Dhawan 2017). The better access to irrigation water through rainwater harvesting result in multiple benefits (Joshi *et al.* 2008), such as:

- (i) Increasing agricultural production.
- (ii) Enhancing cropping intensity and enable two to three crops in a year.
- (iii) Improving groundwater availability.
- (iv) Facilitating crop diversification towards high-value crops.

- (v) Generating employment opportunity.
- (vi) Raising farm incomes.
- (vii) Improving sustainability of soil and water resources.

Joshi et al. (2008) did a meta-analysis of a large number of watersheds to quantify their impact on rainfed agriculture. The results reveal that watershed programmes not only increase farmers' income, but improve equity and sustainability of natural resources in rainfed areas. This also increases the likelihood of raising farm income through crop diversification and integration of fish, poultry and other enterprises in the farming system.

Despite the enormous benefits, the watershed programmes could not succeed without government intervention. Joshi *et al.* (2008) documented the conditions for success for watershed programmes. These are:

- (i) Necessity of people's participation and involvement of local stakeholders in planning, development and execution is crucial.
- (ii) Need for demand-driven activities of watershed programme rather than supply driven.
- (iii) Active participation of women and landless labour.
- (iv) Develop processes for decentralization of decision making.
- (v) Involvement of elected leaders and village heads.
- (vi) Visible tangible economic benefits.
- (vii) Awareness about the benefits of the programmes and community participation.
- (viii) Need to develop linkages with other institutions like credit sector and technology.
- (ix) Implement agro-eco-region specific technologies.

The key messages that emerge from the above review are summarized as follows:

- (i) There are positive economic, social and sustainability impacts due to the adoption of technologies related to natural resource management.
- (ii) There is a large variation across states or agro-eco-regions in the adoption of technologies related with natural resource management.
- (iii) The main adoption barriers are poor access to water, lack of technical knowledge, poor extension services, and small and fragmented land holdings.
- (iv) Main drivers of the adoption are the community participation, government subsidy, access to information, and effective co-ordination among farmers.

- (v) Significant benefits of watershed programmes are increasing efficiency, improving equity and enhancing sustainability.

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Chapter: 3**Weed Biology and Ecology****Jitendra Kumar¹ and Uma²**¹Research Scholar, Dept. of Agronomy, R.B.S. College, Bichpuri, Agra (U.P.) India²Asistant Professor, Dept. of Agronomy, R.B.S. College, Bichpuri, Agra (U.P.) India***E-mail: jkyadav.ag@gmail.com****❖ Weed Biology**

Weed biology is the study of the establishment, growth, reproduction, and life cycles of weed species and weed societies/vegetation. Weed biology is an integrated science with the aim of minimizing the negative effects, as well as using and developing the positive effects, of weeds.

Life Cycle - Based on life cycle weeds are classified as annuals, biennials or perennials.

Annuals

Annuals complete their life cycle from seed in less than one year. There are two types: summer and winter annuals. Summer annuals germinate in the spring, mature, produce flowers and seeds and die before fall. Winter annuals germinate in the fall, overwinter in a seedling or rosette stage, mature, produce flowers and seeds, and die in the spring or early summer. Because of the seedling stage, annual weeds are generally easy to control. There are usually few annual weeds present in low bush blueberry fields.

Biennials

Biennials generally complete their life cycle over two years. The first year the seeds germinate and form a basal cluster of leaves and a tap root. The plant overwinters in this stage. During the second year the weed produces a flower stalk, sets seed and dies. Examples of biennial weed are evening primrose and wild carrot.

Perennials

Perennial weeds live for more than two years. These weeds are the most common in blueberry fields and generally the most difficult to control. Perennial weeds may reproduce primarily by seed (daisy); by both seed and roots (sheep sorrel); or primarily by vegetative means (bunchberry). Many perennial weeds grow in the same manner as the blueberry plant. Therefore, many of the production practices that promote blueberry growth (e.g. pruning)

also promote growth of these weeds. Perennials which are low growing and spread vegetative by interconnected underground root systems are the most difficult to control. Perennial weeds growing above the blueberries may be controlled by wiping or spot treatments with registered herbicides. Perennial weeds include both woody and herbaceous species.

Growth Characters

Based on growth characters weeds are classified as grasses, broadleaf weeds, ferns and herbaceous or woody weeds.

Broadleaf

Broadleaf weeds are annual, biennial or perennial plants which generally have two leaves (cotyledons) emerging upon germination. The leaves normally have a branching network of veins and the flowers have distinct petals.

Grasses, Sedges and Rushes

Grasses can be annual or perennial plants. They generally have narrow, upright, parallel veined leaves. Grasses have jointed stems, usually hollow at the internodes and are circular in cross section. Sedges are a large group of perennial (rarely annual) grass-like plants which are common in wet, poorly drained soils. Sedge stems are triangular in cross section, solid, and not jointed.

Rushes are annual or perennial plants similar in appearance to sedges with grass-like tufted leaves common at the plant base. Rush stems are hollow, circular in cross section, and not jointed. Like the sedge, this plant is also common in wet areas or poorly drained soil, but is also found in woodland and open field.

Ferns

Ferns are primitive perennial plants that do not produce flowers and seeds. Ferns consist of a leaf or frond, a stalk and an expanded blade which may then be further subdivided several times. Ferns spread by long creeping rhizomes and/or by spores.

Reproductive Strategy

Based on reproductive strategy weeds are classified as seed, vegetative reproduction.

Reproduction by seed

Reproduction by seed is called sexual reproduction. It requires the fertilization of an egg by sperm, usually in the form of pollen. Pollination of the egg in a flower results in seed that is capable of producing a new plant. Seed production varies greatly among and within weed species in part due to environmental variability between years, competition from plants, and genetic variability.

Through sexual reproduction abundant and small seeds are produced. Annual and biennial weeds depend on seed production, as the sole means of propagation and survival of perennial weeds are less dependent on this mechanism. For example, while Canada thistle has been observed to produce as few as 680 seeds per plant, curly dock often produces more than 30,000 seeds per plant.

The seed production capacity of some of the weeds is

Ontogeny	Seeds/plant	Name of weed/crop	Seeds/plant
Annuals	20,832	<i>Chenopodium album</i>	72,000
Biennials	26,600	<i>Solanum nigrum</i>	1,78,000
Perennials	16,629	<i>Amaranthus retroflexus</i>	1,96,405

A few weeds may produce seed through apomixes *i.e* without fertilization. Eg. Ferns reproduce by spores.

Vegetative Reproduction

In vegetative (asexual) reproduction, a new plant develops from a vegetative organ such as a stem, root, or leaf. Several modifications of these organs are common in perennial weeds, such as underground stems (rhizomes), above-ground stems (stolons), bulbs, corms, and tubers. Although vegetative structures generally do not survive as long in the soil as do seeds, very small structures can result in a new plant. Vegetative reproduction can be as prolific as seed production.

❖ Weed Ecology

Weed ecology is the study of the interaction or relationship between a weed and its environment (other living organisms as well as abiotic factors). Ecology is concerned with growth characteristics and adaptations that enable weeds to survive the change in the environment. Man plays an important role in changing the environment by altering the crop husbandry practices and by maintaining weed free monocrop or multicrop culture. For

effective weed control, the study on both biology and ecology of a weed species are important.

The weed seedbank and seed dormancy

Not only can weed seed and vegetative tissue travel great distances to infest new fields, but once in the soil, weed seed can remain viable for many years. In any given location, the weed seedbank contains a vast library of weed species and ecotypes that are adapted to a great range of environmental conditions and are ready to germinate given the proper signal. A study reported that a square foot of soil, 6 inches deep, contained from 98 to 3,068 viable weed seeds. This represents between 4.3 million and 133 million viable seeds per acre.

Weeds belong to 8 to 10 to the genera, *Amaranthus*, *Anagallis*, *Avena*, *Chenopodium*, *Convolvulus*, *Digitaria*, *Eleusine*, *Fumaria*, *Lactuca*, *Phalaris*, *Portulaca*, *Solanum* and *Xanthium* were effectively controlled. However, weeds having underground rhizomes (*Cynodon dactylon*), underground tubers (*Cyperus rotundus*) and hard seed coat (*Melilotus spp.*) were not controlled (Patel *et al.*, 2005).

The amount of time that a seed is capable of producing a seedling, or its viability, varies with weed species. In the extreme, lotus (*Nelumbo nucifera*) seeds found in a Manchurian lakebed were viable after 1,000 years. More commonly, the annual plant jimsonweed (*Datura stramonium*) has over a 90 percent germination rate after 40 years in the soil.

Additionally, many weed seeds remain dormant in the soil until the conditions for germination and survival are appropriate for that particular seed. Dormancy is the seed's resting stage and is the primary method of weed seed dispersal in time. Some weed seeds have seed coats that are impermeable to water and/or oxygen or are mechanically resistant. Others contain immature embryos or have a waiting period (called after-ripening) that must be completed before the seed will germinate. Seed dormancy is affected by environmental conditions, including temperature, light, oxygen, and the presence of chemical inhibitors.

Seed dormancy as survival mechanism

Weed seeds possess a variety of special germination mechanisms adapted to changes in temperature, moisture, aeration, exposure to light, depth of burial of seeds etc., when conditions are unfavorable for germination, they can remain dormant or delay germination. Conditions favourable for weeds seed germination are

- Seeds of many weeds require an exposure to light for germination. This is regulated by bluish-green protein pigment called phytochrome.
- Many weed seeds germinate under aerobic conditions while some require anaerobic condition. Soil turnover during ploughing and other operations exposes the seeds to light and induces germination.
- Periodicity of germination is another specialized germination mechanism. *Amaranthus spp.* have a definite pattern of peaks of germination at regular intervals.
- Summer annuals favour higher temperature & winter annuals germinate at lower temperatures some weeds germinate freely throughout the year.

Seed Dormancy

Dormancy is a state of seeds and buds in which they are alive but not germinated. If all weed seeds were to germinate at one time, their seedlings could be destroyed. Dormancy allows storage of millions of weed seeds in soil and enables them to grow in flushes over years. In this context, the old gardeners saying “**One year Seeding seven years Weeding**” is very appropriate. In fact, weed seeds have been found viable even after 20-80 years of burial in soil. Weed seeds exhibit three types of dormancy.

Persistence of Weeds

Weeds are highly persistent category of plants. They existed even before the first seed of any crop was planted on earth and they are with us even today, probably in larger number and with greater vigour. This speaks ample of their highly persistent nature. If a mixed growth of a crop and weeds is left to nature, soon there will be weeds all over, with no trace of the crop plants. High persistence of weeds results from their multi facet mechanisms. Important among these are:-

(i) Prolific Seed Production

Most weeds are prolific seed producers. For instance, the per plant seed production capacity of *Cuscuta spp.* was found approximately 16,000; *Conyza* 33,992; *Chenopodium album*, 72,000; and *Amaranthus*, 196,000. Only in few instances of some perennial weeds, the seed production was weak. Further, the immediate viability of weed seeds has been found to vary from 6 to 78%. Thus, one can anticipate that in majority of cases even if a few weedy plants escaped control measures in the field, they were sufficient to produce enough seeds to continue their progeny for years to come. It is further interesting to note that weeds can set

viable seeds even when they are harvested before full maturity. Perennial sow thistle (*Sonchus arvensis*) can set viable seeds even when it is cut during its flowering stage and kept in shade. Chickweed (*Stellaria media*) and purslane (*Portulaca oleracea*) have also been found well adapted to premature flowering and seed setting under adverse environment conditions. A weedy plant must produce some seeds before it perishes under any stress of nature, may it be drought, biotic pressure, disease or insect pest, etc. It is commonly observed that under favourable conditions *Chenopodium album* may grow as much as 30-50 cm tall before it flowers and sets seeds. But in the events of severe drought it may grow hardly 3 cm high, and still produce some seeds before it withers.

(ii) Dormancy of Weed Seeds and Other Propagules in Soil

As stated earlier, the majorities of weed seeds lying below about 5 cm soil depth remain dormant, and act as source for future flushes of weeds. Permanent experiments laid out for up to 10 decades have shown that depending upon the weed species, the weed seeds retained viability for 2 to 100 years, to different levels. Thus, the soils, both agricultural and otherwise, acted as “reserve banks” for the weed seeds and their other propagules. The phenomenon of dormancy and consequent longevity of weeds seeds in soils is a very important tool with weeds to ensure their everlasting existence against all odds of nature (and man). If all weed seeds present in the soil were to germinate at one time, a single tillage operation could wipe these out of the scene. But contrary to it, only a fraction of weed seeds present in any soil germinate at any time, leaving others to germinate later. Also, a few weedy plants which escaped attention in a field are often sufficient to recoup the seed banks in the soil. In this context, the old gardener’s saying. **“One year seeding is seven year’s weeding”**, still holds good. Van Acker *et al.*, (1993) defined the critical period of weed control (CPWC) is the period during which the crop must be kept weed free to prevent yield loss due to weed infestation. The potential yield of crops can be maximized if the weeds are controlled during the critical period of crop weed competition (Mishra, 1997; Tiwari, 1999).

Within the soil, weed seeds can remain dormant for three reasons, as follows:-

(a) Enforced dormancy

Enforced dormancy in weed seeds is due to their placement deeper than 5 cm, resulting usually from tillage of the field. Weed seeds under this kind of dormancy germinate readily whenever these are restored to the top 3 to 5 cm layer of soil by tillage, provided adequate soil moisture and congenial temperatures were available in this zone of the soil. Enforced

dormancy is a non-specific character of the seeds and it is caused by the absence of red light (r) under the ground, which otherwise induces germination in seeds by activating their phytochrome system (P), comprising a responsive chromophore blue pigment attached to the protein molecule in seeds. Far-red light (fr) deactivates the system and thus induces dormancy in seeds.



Cultivation counters enforced dormancy by bringing the weeds to surface where they are exposed to full sun-light spectrum, besides better aeration. Higher soil temperature and NO_3 content of surface soil may further help in breaking the enforced seed dormancy.

(b) Innate dormancy

Innate dormancy is a genetically controlled character and it is a feature of specific weed seeds. Innate dormant seeds will fail to germinate even if they were present in the top 3 to 5 cm soil and adequate soil moisture and temperature conditions were provided to them. Innate dormancy usually results for reasons of either hard seed coats, like in *Setaria*, *Ipomoea*, and *Xanthium spp.*, or immature embryos, as observed in *Polygonum*, *Juncus*, and *Eleocharis spp.* In certain weed seeds, particularly those of xerophytic origin, the presence of specific germination inhibitors is responsible for their innate dormancy. In nature the innate dormancy of weed seeds is overcome with either passage of time, or under the influence of some climatic pressure.

(c) Induced Dormancy

Induced dormancy results from some physiological change in otherwise non-dormant weed seeds under the impact of factors like a marked rise in soil temperature, increased CO_2 content of the soil, low O_2 pressures, water logging, etc. Certain weed seeds, like those of wild oat (*Avena fatua*), exhibit all the three kinds of dormancies.

(iii) Vegetative Propagation

Many weeds are extremely persistent because of their ability to propagate by vegetative means. When the above ground parts of such weeds are destroyed, their deeply placed

vegetative propagules put forth new shoots as soon as the external stresses are removed and favourable conditions revive. Even if some kind of deep tillage is employed in an attempt to destroy these, it will be a futile exercise since fragments of the weed propagules get easily dispersed to new areas where they initiate fresh colonies.

(iv) Rapid Dispersal

Dispersal is a very important means of persistence of weeds. It exposes them to different ecosystems so that each weed species can choose its most favourable environment and put up a hard struggle for existence in nature. Dispersal is the key factor in developing persistent weed population for every possible niche in the environment. Dispersal aids persistence of weeds in proportion to the dispersal agents and adaptations available to them.

(v) Inherent Hardiness

To be widely successful a weed species must adapt itself to diverse environmental conditions. Weeds seem to possess some kind of built-in mechanism to survive against the vagaries of nature, like extreme cold, heat, drought, biotic stress, and soil abnormalities. This is inherent hardiness of weeds. It is hard to find weeds damaged under a night's severe frost, or destroyed because of attack by some insect pest, disease organism, or nematode. Weeds are rarely seen to either develop chlorosis or any other mineral deficiency or toxicity symptoms, even though the soil may be actually abnormal in its mineral composition. Also, poor quality water declared unfit for irrigating crops, fails to injure weeds. In fact, in crop fields all abnormal features of soil and water help weeds to grow vigorously in spaces vacated by the stunted growth (or death) of the crop plants. The mechanisms involved in this inherent hardiness of weeds are not much known. Further, several weeds of tropical origin, e.g. *Cyperus* and *Amaranthus spp.*, have the advantage of adopting C₄ pathway of CO₂ fixation, which is devoid of photorespiration. Several weeds have exceptionally high transpiration efficiency, low nutrient requirements, and slow rates of translocation of food and minerals. Such adaptations in weeds help them to persist in adverse weather conditions. High rates of elongation in certain weeds, particularly in the initial stages of growth, improve their competing ability and persistence. But a common aspect of inherent hardiness of all weeds seems to be their numerous generations of natural selection through climatic stresses in the struggle for survival of the fittest.

(vi) Evasiveness

Many a weed is capable of evading destruction by animals and man because of their bitter taste, disagreeable odour, spiny nature, and mimicry.

(vii) Self Regeneration

Weeds are self-sown plants. They don't require any artificial, friable seedbeds for their germination. Detached from the mother plants, weed seeds and other propagules germinate profusely on undisturbed soils whenever the environment is favourable for the purpose.

(viii) Selective Invasion

Weed species differ widely in their soil and climatic requirements. But in the first instance, the weed flora composition depends upon the chance a particular weed had to reach a particular site. The nature then makes a critical selection out of the lot and allows only those weed seeds to germinate at a time which were most adapted to the environment prevailing then. The seeds of rest of the weed species wait in soil till the environment outside changes. At this stage, suddenly another set of weed species takes over the ground. The major environment factors which determine the weed species composition on the ground comprise the available soil moisture, soil pH, temperature, photoperiod, and solar energy.

(ix) Weed Succession:

In nature, the individuals of a weed species often have chance to cross breed to variable levels. This leads to the development of a few plants of different genetic make-up, forming new races within a species. Such races of weeds are called agricultural ecotypes. When herbicides (or bioagents) are used continuously to destroy the "normal" races of a weed species, their newly developed ecotypes may sometimes prove tolerant to the herbicides used whence they get chance to gradually expand in numbers, each season.

Finally, the overall persistence of a weed depends upon its capability to adopt one or more of the above cited features. A weed species that embodies majority of these factors is surely a horrible weed, for instance, *Sorghum halepense* and *Saccharum spontaneum*.

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Chapter: 4
Development of Hybrid Time Series Models for Forecasting Total Rice Production in Assam

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Abstract: The study of forecasting in time series analysis has become a powerful tool in different applications in the agricultural field. The major goal of time series models comprises forecasting into the short- and long-term period, smoothening of irregular series and causal modelling of variables moving with time. Yearly data on production of Total rice have been used for forecasting from the year 1951 to 2018. The data from 1951-1998 were used for model building and 1999 - 2018 were used for checking the forecasting performance of the model. In this study ARIMA, ANN, SVM time series models and hybrid of both ARIMA-ANN, ARIMA-SVM were used to analyse the past behaviour of production of Total rice in order to make inferences about its future behaviour. ARIMA (1,1,0) model was selected as suitable model for Total rice. For training data set MAE for hybrid ARIMA (1,1,0)-SVM was found to be 89141.216 as compare to 90045.612 of ARIMA-ANN; 117942.833 of ARIMA (1,1,0); 93728.551 of ANN; 91131.651 of SVM. For testing data set, MAE for hybrid ARIMA (1,1,0)-SVM was found to be 11097.031 as compare to as compare to 12131.450 of ARIMA-ANN; 249819.618 of ARIMA (1,1,0); 147083.319 of ANN; 13912.415 of SVM. Hence, the performances of hybrid ARIMA-ANN and ARIMA-SVM were found to be better than that of ARIMA for both under training as well as testing data sets. And from the results, we found hybrid approach gives better results for forecasting of crop production.

1. Introduction:

Statistical forecasting model is used to develop accurate forecasting by using past data through identification of trends and patterns of the data. In other words, statistical forecasting is the likelihood estimation of an event to take place in future based on available data. Proper forecast is very much essential in an economic system as it would be easier to formulate the policy makers regarding allocation, price fixation, procurement, irrigation, marketing and storage. Statistical computing, modelling and forecasting data are key instruments for addressing these problems. The study of forecasting in time series analysis has become a powerful tool in different applications in the agricultural field. The major goal of time series

models comprises forecasting into the short- and long-term period, smoothening of irregular series and causal modelling of variables moving with time. Dependency through time is the basis for extrapolation into the future. Another goal of time series analysis is detecting changes in structure in the series- possibly as a result of an intervention such as economic policy, pollution incident or medical treatment.

One of the major and widely used time series models is the autoregressive integrated moving average (ARIMA) model. Due to its statistical properties, ARIMA model is more popular in time series data. It is also known as Box-Jenkins methodology (Box and Jenkins 1970) in the model building process. But this model has a limitation as it assumes linearity as observed in the time series. Therefore, no nonlinear patterns can be detected by the ARIMA model. However, linearity is a useful assumption and powerful tool in many different areas. It became clearer in the early 1980s that the approximation of the linear models for complex data is not always satisfactory. The last two decades plenty of literature have emerged that deal with testing and modelling of non-linear time series data. The recent resurgence of research activities in Artificial Neural Network (ANN) as well as its successful applications suggest that it can be an important method for time series forecasting. As compared to the traditional methods, ANN is a data driven, self-adaptive, nonlinear, nonparametric method in which there are a few a priori assumptions. The major advantage of neural network (NN) is flexibility of nonlinear modelling and no need to specify a particular model form.

Neural networks and traditional time series models have been compared in several studies. Kohzadi et al. revealed that the neural networks are superior to ARIMA methods for forecasting of commodity price. Hervai et al. examined linear and ANN models for forecasting seasonally unadjusted monthly data on European industrial production series and conclude that linear models generally produce more accurate post sample forecast than neural network models at horizons of up to a year, in terms of root mean sq. error.

Generally, agricultural data known to be multifaceted and often nonlinear in nature, so in this study ARIMA, ANN, SVM time series models and hybrid of both ARIMA-ANN, ARIMA-SVM were used to analyse the past behaviour of production of agricultural crop in order to make inferences about its future behaviour. It has been observed that hybrid methods (Zhang 2003, Jha and Sinha 2014) are more effective and efficient ways to improve forecast ability of the model.

2. Data and methodology:

Yearly data on production of Total rice have been used for forecasting from the year 1951 to 2018. The data from 1951-1998 were used for model building and 1999 - 2018 were used for checking the forecasting performance of the model. The statistical software viz., SPSS, R; were used for modelling and forecasting of production of agricultural crops in Assam. SPSS software was used to build the suitable ARIMA model nonlinearity test for residuals obtained from ARIMA models using Ljung-Box test. R software package 'Forecast' was used for modelling and forecasting using NN and package 'e 1071' was used for modelling and forecasting using SVM.

ARIMA models represent different kinds of time series as AR, MA, and ARMA series [Zhang 2003]. ARIMA models are flexible in nature and have more powerful and efficient than other structural models to generate short run prediction.

2.1 Time series forecasting models:

2.1.1 The ARIMA model:

In an ARIMA model, time series variable is assumed to be a linear function of past actual values and random shocks. An ARIMA (p, d, q) model is defined by the following equation

$$\phi(B)(1-B)^d y_t = \theta(B) \varepsilon_t \quad [1]$$

Where,

$$\phi(B) = 1 - \phi_1 B - \phi_2 B^2 - \dots - \phi_p B^p \quad (\text{Autoregressive parameter})$$

$$\theta(B) = 1 - \theta_1 B - \theta_2 B^2 - \dots - \theta_q B^q \quad (\text{Moving average parameter})$$

ε_t = White noise or Error term

d = Differencing term

B = Backshift operator i.e., $BaY_t = Y_{t-a}$

ARIMA methodology is carried out in three stages, Viz., Identification, estimation and diagnostic checking. Identification of d is necessary to make the non-stationary time series to stationary. A formal statistical test to check the stationarity, known as the test of unit root hypothesis or Augmented Dickey Fuller test. ADF test was utilized to check the stationarity. A good account on ADF test can be found in Makridakis et al. (1998). At the stage of estimation, parameters are estimated for the ARIMA model tentatively chosen at the early identification stage. Parameters estimation for ARIMA model is generally done through iterative least squares method. The adequacy of selected model is tested at the stage of diagnostic checking. At this stage, testing is done to see if the estimated model is statistically adequate i.e., whether the error terms are white noise which means error terms are uncorrelated with mean zero and constant variance. For this purpose, Ljung-Box test is applied to the original series or to the residuals after fitting a model. A good account on

Ljung-Box test can be found in Box et al. (1994). If the model is found to be inadequate, the three stages are repeated until satisfactory ARIMA model is selected for the time series under consideration.

2.1.2 Ljung-Box test :

The Ljung-Box test, named after statisticians Greta M. Ljung and George E.P. Box, is a statistical test that checks if autocorrelation exists in a time series. It is sometimes called Box-Pierce test. The test identifies whether or not errors are iid (i.e., white noise). The null hypothesis of Ljung-Box test is

H_0 : The residuals are independently distributed and the alternative hypothesis is

H_1 : The residuals are not independently distributed; they exhibit serial correlation.

The test statistic for the Ljung-Box test is as follows:

$$Q = n(n+2) \sum p_k^2 / (n-k); \text{ where:}$$

n = sample size

p_k = sample autocorrelation at lag k

The test statistic Q follows a chi-square distribution with h degrees of freedom; that is,

$$Q \sim \chi^2(h).$$

We reject the null hypothesis and say that the residuals of the model are not independently distributed if $Q > \chi^2_{1-\alpha, h}$

2.1.3 Artificial Neural Network (ANN) model:

ANN(s) models are set of nonlinear models that are able to capture different nonlinear structures present in the data set. The specification of ANN model does not require any prior assumption of the data generating process, instead it is largely depended on characteristics of the data known as data-driven approach. Single hidden layer feed forward network is the most widely used model for time series modelling and forecasting. This model is constructed by a network of three layers of simple processing units, and thus termed as multilayer ANNs. The first layer is input layer, the middle layer is the hidden layer and the last layer is output layer.

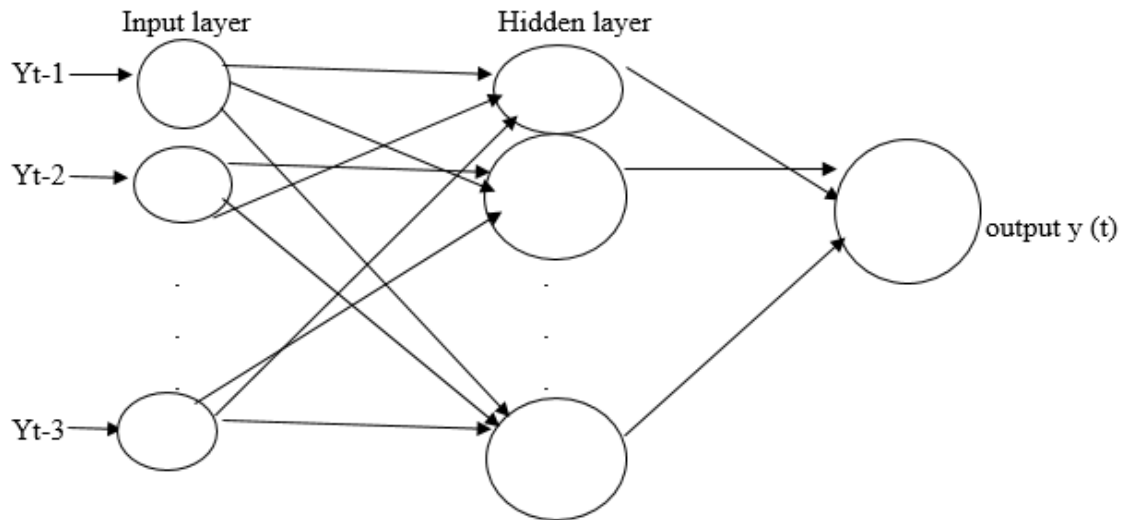


Figure 2.1: Neural Network architecture

The relationship between the output (y_t) and the inputs ($y_{t-1}, y_{t-2}, \dots, y_{t-p}$) can be mathematically represented as follows:

$$Y_t = f \left(\sum_{j=0}^q w_j g \left(\sum_{i=0}^p w_{ij} y_{t-i} \right) \right) \quad (1)$$

Where $w_j (j=0,1,2,\dots,q)$ and $w_{ij} (i=0,1,2,\dots,p; j=0,1,2,\dots,q)$ are the model parameters often called the connection weights; p is the number of input nodes and q is the number of hidden nodes, g and f denote the activation function at hidden and output layer respectively.

2.1.4. Support Vector Machine:

Support vector machine proposed by Vapnik (1998) is a nonlinear algorithm used in supervised learning framework for data classification, pattern recognition and regression analysis. The model has been built in two steps: i.e., training and testing. In the training and testing. In the training step, the largest part of the dataset has been used for the estimation of the function. In the testing step, the generalization ability of the model has been evaluated by checking the model performance in the small subset.

It has been used in a wide range of applications such as in data mining, classification, regression and time series forecasting (Cao and Tay, 2001; Flake and Lawrence, 2002; Zhao et al. 2006). The ability of SVM is to solve nonlinear regression estimation problems and it makes SVM successful in time series forecasting.

The SVM architecture is shown in the following Figure.

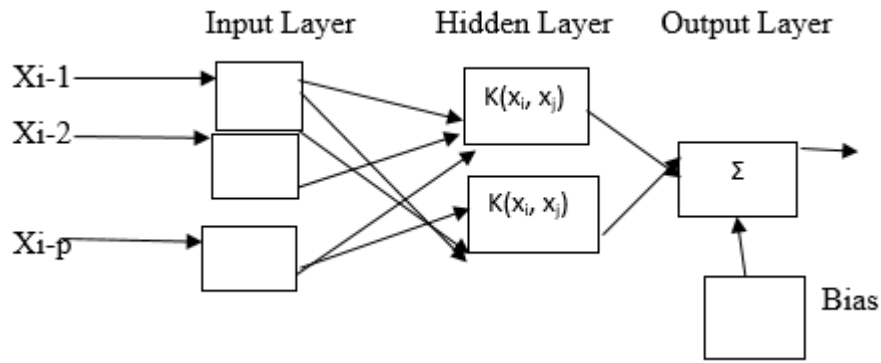


Figure.2.3: SVM Architecture

Evaluation Criteria:

The most common error function in neural networks is the sum of squared errors. Other error functions offered by different software include least absolute deviations, least fourth powers, asymmetric least squares and percentage differences.

2.1.5. Hybrid approach:

The proposed approach considered time series (y_t) as a function of linear and non-linear components. Hence $y_t = f(L_t, N_t)$

where y_t is a time series data; L_t and N_t represents the linear and nonlinear component respectively. This approach follows the Zhang's (2003) hybrid approach, accordingly the relationship between linear and nonlinear components can be written as following

$$Y_t = L_t + N_t$$

The main strategy of this approach is to model the linear and nonlinear components separately by different model. The methodology consists of three steps. Firstly, ARIMA model is applied to the data series to fit the linear part. Let the prediction series provided by ARIMA model denoted as $\hat{L}_t$. In the second step, instead of predicting the linear component, the residuals denoted as e_t which are nonlinear in nature are predicted. The residuals can be obtained by subtracting the predicted value $\hat{L}_t$ from actual value of the considered time series y_t .

$$e_t = y_t - \hat{L}_t$$

Now the residuals are predicted employing an ANN and SVM model. Let the prediction series provided by ANN/SVM model denoted as $\hat{N}_t$. Finally, the predicted linear and nonlinear components are combined to generate aggregate prediction.

$$\hat{y}_t = \hat{L}_t + \hat{N}_t$$

Ljung-Box test is used to test for non-linearity in this study.

The graphical representation of proposed approach is expressed in the figure 2.2 & 2.3

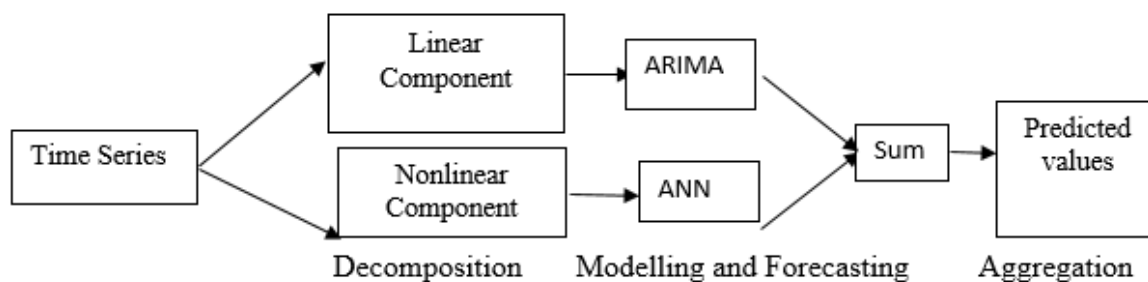


Figure 2.2: Schematic representation of ARIMA-ANN hybrid methodology

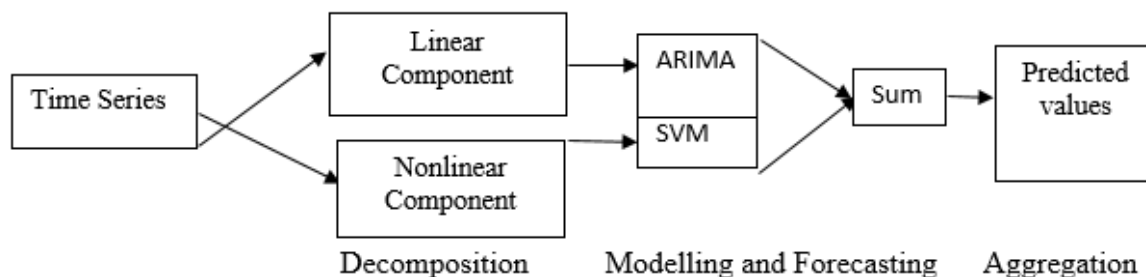


Figure 2.3: Schematic representation of ARIMA-SVM hybrid methodology

Forecasting Performance:

Forecasting Performance of the model has been adjusted by computing mean absolute error (MAE). The model with minimum values of MAE for training and testing data set is preferred for forecasting purpose. The MAE is computed as

$$MAE = \frac{1}{n} \sum_{t=1}^n |y_t - \hat{y}_t|$$

Where n is the total number of forecast values. y_t is the actual value at period t and $\hat{y}_t$ is the corresponding forecast value.

3. Results and Discussion:

Table 3.1: Summary Statistics of time series for Production of Total rice

Statistic	Total rice Production	Statistic	Total rice Production
Observation	68	Maximum	5437372
Mean	2861250.088	Standard deviation	1188160.938
Median	2496103.5	Skewness	0.731
Range	4039179	Kurtosis	-0.564
Minimum	1398193	Coefficient of	240.81

variation (%)

Here we have applied hybrid ARIMA approaches using ANN and SVM on production of selected crops from the year 1951 to 2018 whereas from 1951 to 1998 as a training set and 1999 to 2018 as a testing set. Sequence charts for production of Total rice is shown in Figure 3.1

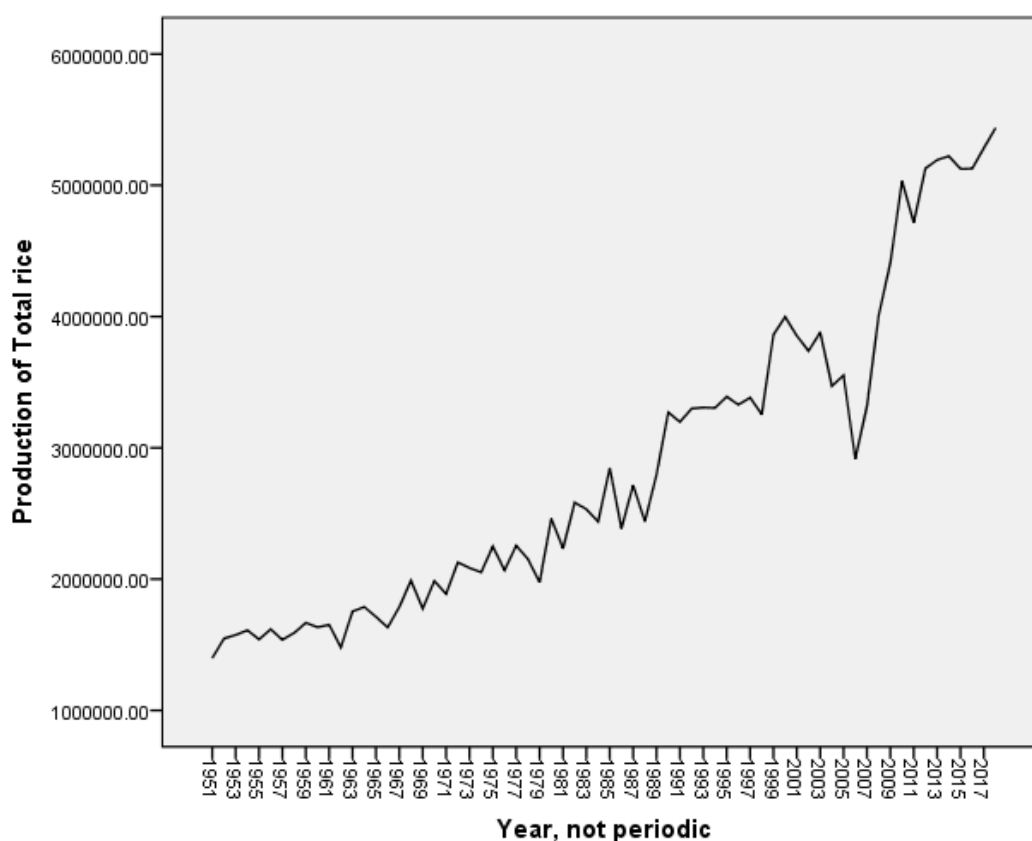


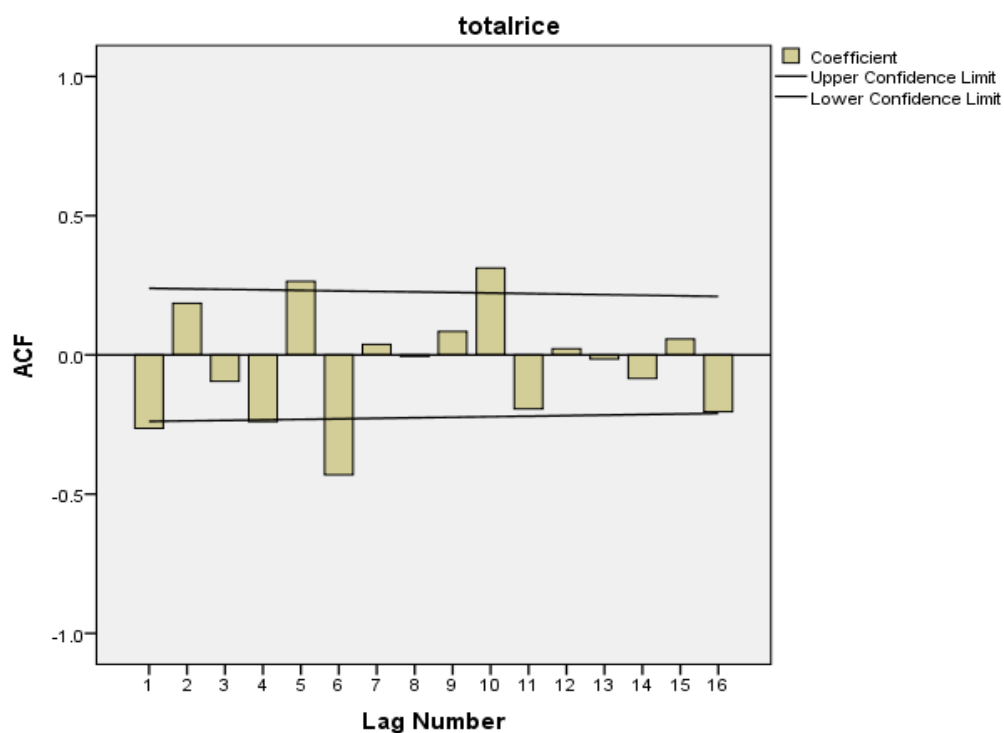
Figure 3.1: Sequence charts for production of Total rice

3.2. Forecasting through hybrid univariate linear time series approach

For linear time series models, Auto Regressive Integrated Moving Average (ARIMA) method was applied on the production of Autumn rice from the period 1951-52 to 2018-19 for forecasting over Assam. Based on the minimum values of measures of goodness of fit, like Root Mean Square Error (RMSE), Mean absolute Percentage Error (MAPE), Mean Absolute Error (MAE) given in Table 3.2 (BIC= 24.952), white noise test using Ljung-Box Q test for residuals reported in Table 3.3 (p value= .049) and significance of the parameter estimates given in Table 3.4, we selected the model ARIMA (1,1,0) as a best fitted for the production of total rice and the same model was found by auto.arima option from R software. By using

the linear model ARIMA (1,1,0), we forecasted production value by 2025 which are given in Table 3.5 and graphically presented in the following figure 3.4.

BDS test was applied to checked the residuals obtained through ARIMA (1,1,0) supports that the residuals are nonlinear because $p < 0.000$, hence we can apply artificial neural network (ANN) approach for modelling and forecasting of residuals. ANN and SVM approaches were applied on the residuals obtained through the selected model ARIMA (1,1,0). For better performance of forecasting of production of total rice, we applied hybrid ARIMA approach using ANN and SVM. We have tried different neural networks with different time delays with different hidden nodes on residuals and the results are presented in Table 3.6.



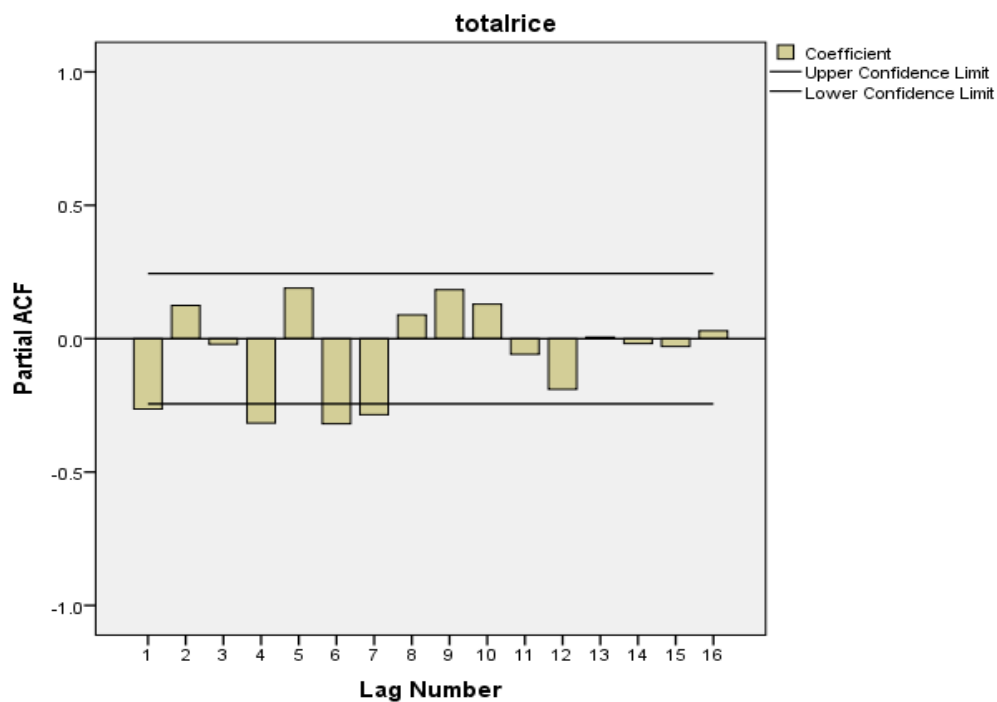


Figure 3.2: ACF & PACF for production of total rice

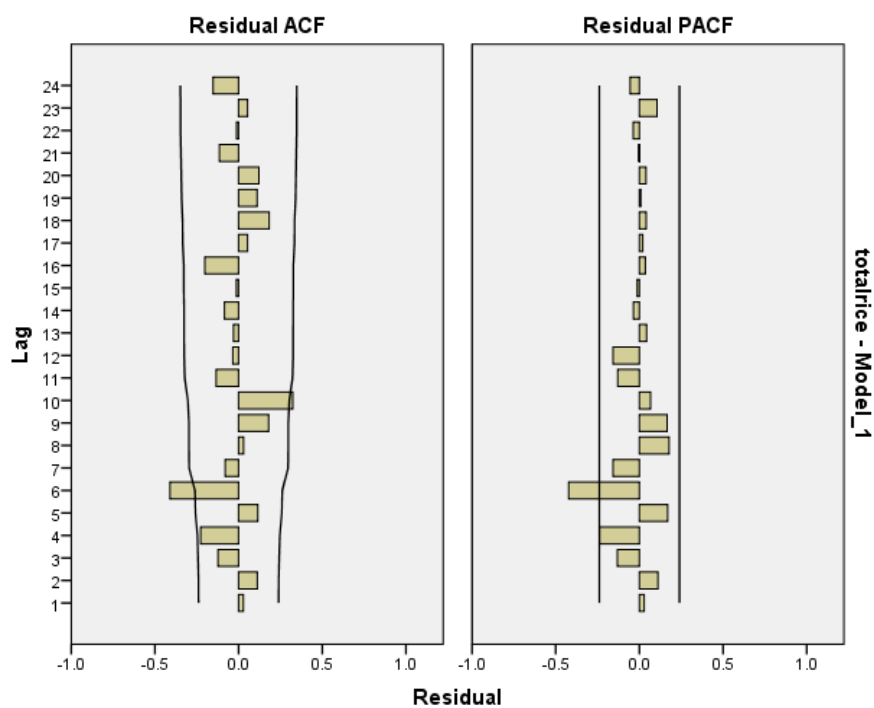


Figure 3.3: Residual ACF & Residual PACF for production of total rice

Table 3.2: Goodness of fit Statistics of Total rice

Fit Statistic	ARIMA (0,1,1)	ARIMA (1,1,0)	ARIMA (1,1,1)	ARIMA (2,1,2)
Stationary R-squared	0.056	0.070	0.079	0.247
R-squared	0.957	.957	0.958	0.966
RMSE	247888.458	246016.518	246756.110	226664.863
MAPE	6.607	6.491	6.485	6.160
MAE	181167.902	178823.211	178933.897	167620.822
MaxAPE	24.442	23.676	22.687	17.751
MaxAE	712724.161	719635.687	758948.001	679576.205
Normalized BIC	24.967	24.952	25.021	24.976

Table 3.3: Test for white noise of Total rice

Model	Ljung-Box Q		
	Statistics	DF	Sig.
ARIMA (0,1,1)	40.464	17	0.001
ARIMA (1,1,0)	40.628	17	0.049
ARIMA (1,1,1)	41.538	16	0.000
ARIMA (2,1,2)	23.780	14	0.001

Table 3.4: Parameter Estimates of Total rice

Models	Parameter Estimate	SE	t	Sig.
ARIMA (0,1,1)	0.215	0.121	1.771	0.081
ARIMA (1,1,0)	-0.261	0.120	-2.181	0.033

ARIMA (1,1,1)

AR Lag1	-0.486	0.385	-1.261	0.212
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MA Lag1	-0.234	0.429	-0.546	0.587
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ARIMA (2,1,2)

AR Lag1	-1.547	0.053	-29.057	0.000
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AR Lag2	-0.962	0.051	-18.948	0.000
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MA Lag1	-1.482	0.953	-1.554	0.125
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MA Lag2	-0.997	1.279	-0.780	0.438
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Table 3.5: Forecast of production of Total rice

Year	Forecast	LCL	UCL
2019	5472561.91	4981267.34	5963856.48
2020	5538684.90	4927825.09	6149544.71
2021	5596731.05	4868466.55	6324995.55
2022	5656886.12	4831822.72	6481949.52
2023	5716490.54	4803875.48	6629105.59
2024	5776238.73	4783999.81	6768477.66
2025	5835949.39	4769961.01	6901937.76

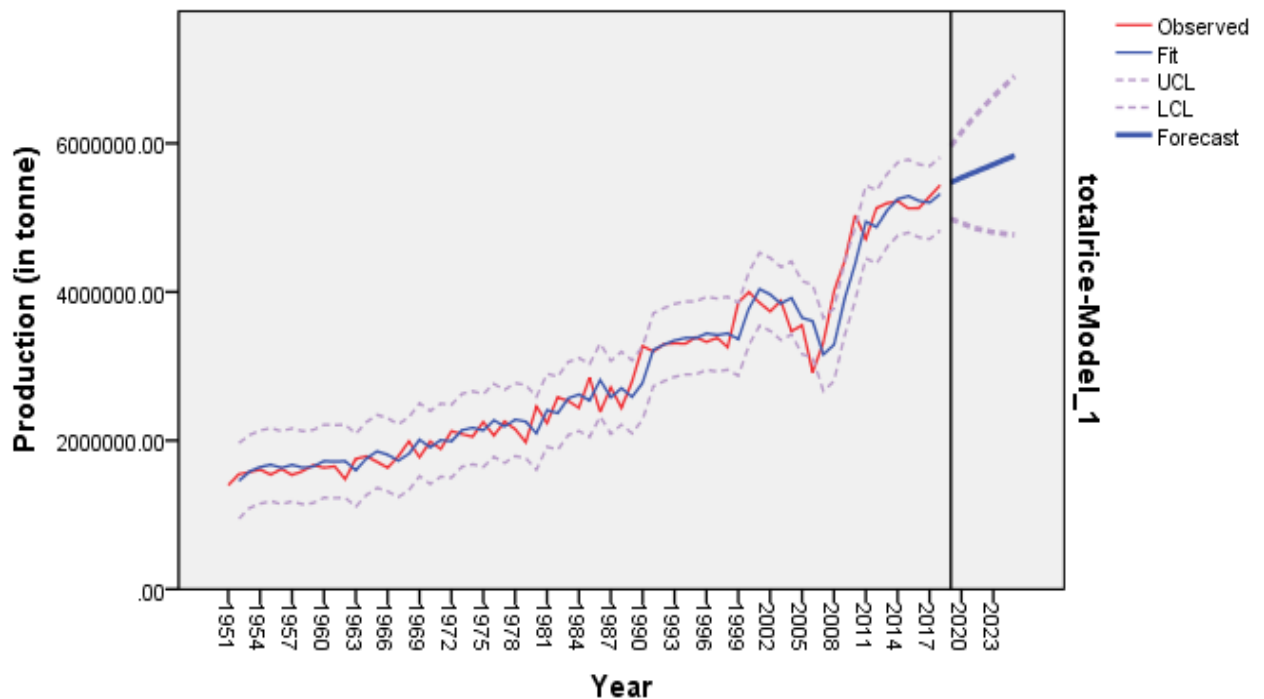


Figure 3.4: Forecast of production of total rice by 2025 using ARIMA (1,1,0)

For Neural Network, we have used different combinations of models with different time delays and hidden nodes on the residuals and the results are displayed in Table 3.6.

Table 3.6: MAE for Neural Network models for production of Total rice

Model parameters	MAE for Training	MAE for Testing
1:2s:1l	115185.460	253415.812
1:4s:1l	115152.154	251080.920
1:6s:1l	114888.638	252271.346
1:8s:1l	115548.877	251074.906
1:10s:1l	115476.832	250776.060
2:2s:1l	115654.361	264901.060
2:4s:1l	115520.565	266354.041
2:6s:1l	115554.108	265357.932
2:8s:1l	116924.211	263190.248
2:10s:1l	115863.417	263617.694

3:2s:1l	116903.610	248277.295
3:4s:1l	116888.879	249140.068
3:6s:1l	117816.662	249551.253
3:8s:1l	118108.000	247974.037
3:10s:1l	118614.207	249056.756
4:2s:1l	117171.536	245359.128
4:4s:1l	116544.639	241409.559
4:6s:1l	116688.447	252135.720
4:8s:1l	117994.658	247588.938
4:10s:1l	118111.808	239957.986
5:2s:1l	113735.006	248430.014
5:4s:1l	114939.364	240565.884
5:6s:1l	113264.519	243100.484
5:8s:1l	113919.223	246874.591
5:10s:1l	111942.130	241444.102
6:2s:1l	93437.742	172561.660
6:4s:1l	93728.551	147083.319
6:6s:1l	96147.205	139503.662
6:8s:1l	96970.231	139377.209
6:10s:1l	97905.311	139521.167

From the above table, the model 6:4s:1l was found to be the best one on the basis of minimum values of MAE for training=93728.551 and testing= 147083.319. From this selected model we have got the estimated values of residuals and fitted values of production of total rice obtained by ARIMA (1,1,0) then forecast value of production was obtained through hybrid approach i.e., ARIMA (1,1,0)-ANN. The goodness of fit measure MAE for hybrid ARIMA-ANN was found to be 169431.067 as compare to 178823.211 of ARIMA

(1,1,0). Residuals obtained by using ARIMA (1,1,0) were applied on the non-linear approach support vector machine using radial basis function as kernel. Forecast values of production obtain through ARIMA (1,1,0) were corrected by using the residuals through SVM and estimated the value MAE for hybrid ARIMA-SVM. MAE for hybrid ARIMA-SVM was found to be 163583.607 as compare to 178823.211 of ARIMA (1,1,0) and 169431.067 of hybrid ARIMA-ANN. Hence the performance of hybrid model found to be better than ARIMA (1,1,0) alone.

For the purpose of forecast value of production through hybrid approach, we have got forecast of residuals through the best neural model (06:4s:1l) till 2025. On the basis of the forecasted value of residuals we found the forecast value of production through hybrid approaches and presented in Table 3.7 along with forecast values by ARIMA (1,1,0).

Table 3.7: Experimental Results of forecast of Production of Total rice

Year	Actual values of Production	Forecast Production by ARIMA (1,1,0)	Forecast Production by Hybrid Approach using ANN	Forecast Production by Hybrid Approach using SVM
1951	1398193.00			
1952	1547726.00	1457911.43		
1953	1574655.00	1583993.24		
1954	1610221.00	1642934.97		
1955	1540933.00	1676245.79		
1956	1617968.00	1634335.87		
1957	1537439.00	1673164.95		
1958	1589974.00	1633776.97	1589117.603	1519405.05
1959	1667477.00	1651568.07	1657111.974	1629405.03
1960	1633002.00	1722551.76	1734401.119	1667362.93
1961	1652239.00	1717314.96	1801564.995	1586599.24
1962	1479139.00	1722527.40	1684262.557	1634619.04

1963	1754511.00	1599647.88	1626170.928	1445127.6
1964	1788325.00	1757920.83	1819206.527	1842720.12
1965	1713354.00	1854807.25	1891875.555	1701629.17
1966	1632438.00	1808240.74	1824152.889	1633439.75
1967	1787512.00	1728877.02	1643031.47	1598737.26
1968	1989654.00	1822332.45	2005019.685	1779378.98
1969	1775552.00	2012184.68	2011384.512	1887516.55
1970	1986719.00	1906766.78	1849189.926	1636731.32
1971	1887410.00	2006893.19	1979963.098	2006978.22
1972	2127303.00	1988651.56	2067233.347	1746214.81
1973	2085438.00	2139976.64	2163161.061	2096445.44
1974	2052055.00	2171680.54	2058559.21	1950545.36
1975	2248988.00	2136082.83	2233240.495	2029201.46
1976	2066981.00	2272878.78	2193943.49	2213569.17
1977	2255966.00	2189815.56	2267981.44	1978574.22
1978	2152825.00	2281932.06	2191343.664	2305811.44
1979	1975380.00	2255067.12	2249358.469	2069803.94
1980	2459893.00	2097023.39	2152934.798	2003465.88
1981	2233123.00	2408694.71	2354684.675	2485605.61
1982	2583253.00	2367645.48	2568756.669	2113034.29
1983	2532314.00	2567143.02	2491178.018	2698411.52
1984	2437949.00	2620925.82	2582913.643	2497194.98
1985	2846544.00	2537899.64	2691122.379	2530893.18
1986	2385274.00	2815168.41	2643804.336	2940369.36
1987	2715847.00	2581026.00	2683120.313	2412487.59

1988	2439303.00	2704843.48	2560042.969	2945643
1989	2794881.00	2586821.80	2557501.605	2451556.52
1990	3270193.00	2777348.51	3057359.686	2959161.2
1991	3197165.00	3221397.15	3101459.274	3296743.49
1992	3299621.00	3291544.41	3565565.713	3272523.85
1993	3307144.00	3348180.36	3153835.546	3322324.59
1994	3304100.00	3380491.00	3401309.746	3335591.64
1995	3390023.00	3380206.12	3327414.153	3311822
1996	3328148.00	3442899.24	3256479.342	3396343.88
1997	3382874.00	3419615.29	3422938.174	3331714.76
1998	3254832.00	3443895.98	3419054.627	3369586.71
1999	3860648.00	3363575.93	3350861.62	3253150.02
2000	3998443.00	3777776.68	3911683.971	3854932.07
2001	3854248.00	4037775.11	4077740.034	4029371.32
2002	3737802.00	3967209.59	3903027.24	3788664.89
2003	3881036.00	3843518.14	3688437.456	3637392.41
2004	3470748.00	3918947.95	4060548.883	3778359.86
2005	3552438.00	3653188.26	3503361.842	3404149.05
2006	2916014.00	3606419.50	3472556.637	3427021.74
2007	3318608.00	3157499.89	3213764.761	2880451.5
2008	4008435.00	3288799.31	3670342.508	3361677.11
2009	4408424.00	3903627.85	4141753.836	4077350.69
2010	5032529.00	4379295.50	4914759.272	4428813.15
2011	4715679.00	4944882.30	4793603.222	5011818.5
2012	5128508.00	4873721.97	4899562.063	4664147.7

2013	5193379.00	5096026.89	4973410.654	5265759.03
2014	5222645.00	5251752.06	5106955.477	5172923.11
2015	5125104.00	5290314.76	5157977.749	5152461.94
2016	5127375.00	5225883.92	4974725.965	5014040.24
2017	5283713.00	5202093.34	5306286.209	5075527.08
2018	5437372.00	5318203.41	5244294	5259825.66
2019		5472561.91	5473465.544	5295231.59
2020		5538684.90	5509907.444	5119744
2021		5596731.05	5589054.359	5164968
2022		5656886.12	5663368.797	5180735
2023		5716490.54	5678891.209	5181103
2024		5776238.73	5704479.887	5180459
2025		5835949.39	5799359.776	5180427

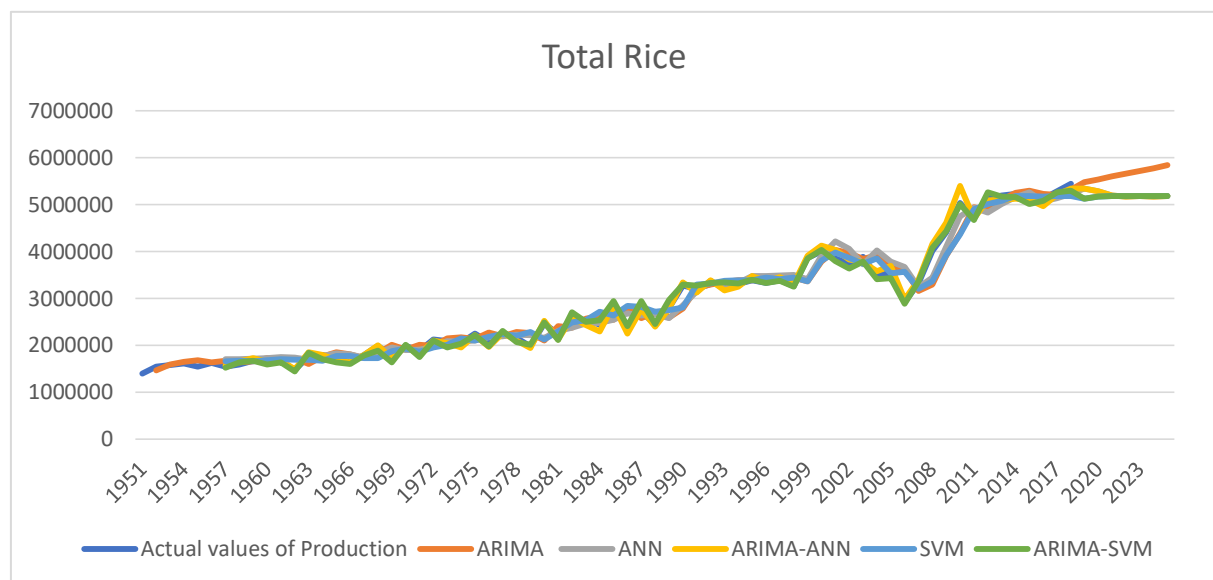


Figure 3.5.: Comparison graphs among ARIMA, ANN, ARIMA-ANN, ARIMA-SVM

Table 3.8: MAE of different models for production of total rice

Data	ARIMA	ANN	SVM	ARIMA-ANN	ARIMA-SVM
------	-------	-----	-----	-----------	-----------

Training	117942.833	93728.551	91131.651	90045.612	89141.216
Testing	249819.618	147083.319	13912.415	12131.450	11097.031

From the above table, the value of MAE under training set for different models ARIMA (1,1,0), ANN (06:10s:1l), SVM, ARIMA-ANN and ARIMA-SVM are found to be 117942.833, 93728.551, 91131.651, 90045.612 & 89141.216 respectively, whereas the value of MAE under testing set are found to be 249819.618, 147083.319, 13912.415, 12131.450 & 11097.031 respectively. Based on these results the model ARIMA-SVM can be recommended for forecasting of production of crop because of the minimum value of MAE both under training and testing set.

4. Conclusion:

In order to enhance the performance of the ARIMA model for forecasting of Total rice production. For this purpose, we have used time series data of Assam from 1951-2018. ARIMA (1,1,0) model was selected as suitable model for Total rice. For training data set MAE for hybrid ARIMA (1,1,0)-SVM was found to be 89141.216 as compare to 90045.612 of ARIMA-ANN; 117942.833 of ARIMA (1,1,0); 93728.551 of ANN; 91131.651 of SVM. For testing data set, MAE for hybrid ARIMA (1,1,0)-SVM was found to be 11097.031 as compare to as compare to 12131.450 of ARIMA-ANN; 249819.618 of ARIMA (1,1,0); 147083.319 of ANN; 13912.415 of SVM. Hence, the performances of hybrid ARIMA-ANN and ARIMA-SVM were found to be better than that of ARIMA for both under training as well as testing data sets. And from the results, we found hybrid approach gives better results for forecasting of crop production.

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Chapter: 5
Common fungal diseases of Millet from all over the world.

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Abstract: A significant grain crop grown in numerous nations worldwide is millet. Bacterial and viral diseases are less severe in pearl millet than fungal diseases. The most detrimental fungal disease to pearl millet in the world is downy mildew, often known as green ear disease, followed by the blast, while rust and leaf spots are less significant. Globally, sorghum is frequently affected by diseases like grain mould, anthracnose, downy mildew, ergot, smut, leaf blight, and leaf spots.

Keywords: Millet, mould, anthracnose, downy mildew, ergot, smut, leaf blight, leaf spots, diseases.

Introduction

Any of the numerous kinds of cereal grasses in the Poaceae family that are grown for their tiny edible seeds is known as millet. Millets were significant cereals in Europe during the Middle Ages and were likely initially farmed more than 4,000 years ago in Asia. They continue to be significant dietary staples in underdeveloped nations worldwide, even though they are now primarily utilised for grazing or to generate hay in the United States and Western Europe. (Britannica-Millet plant 2023)

A significant grain crop grown in numerous nations worldwide is millet. Grain is very nutritive, with high fibre and essential nutrients is very nourishing. Additionally, it is a crop that can withstand drought and be cultivated in dry, arid environments where other crops would fail. Especially in South India, Mali, Nigeria, and Niger, millets are significant crops in the semiarid tropics of Asia and Africa. 97% of millet is produced in poor nations. In recent years, millet output has increased in India.



Figure 1: Millet plant (from Britannica-millet plant)

Bacterial and viral diseases are less severe in pearl millet than fungal diseases. The most detrimental fungal disease to pearl millet in the world is downy mildew, often known as green ear disease, followed by the blast, while rust and leaf spots are less significant (Nagaraja, A., & Das, I. 2016). Globally, sorghum is frequently affected by diseases like grain mould, anthracnose, downy mildew, ergot, smut, leaf blight, and leaf spots (Das, I. 2017).

The common diseases that affect Sorghum include

- **Anthracnose of Sorghum.**
- **Downy mildew of Sorghum,**
- **Ergot of Sorghum or Grain mold of Sorghum,**
- **Leaf blight of Sorghum,**
- **Smut of Sorghum and**
- **Leaf spot diseases**

Anthracnose

One of the most prevalent foliar diseases affecting sorghum is anthracnose, which affects all aerial plant tissues and can reduce seed production by up to 50% in badly damaged fields (Dongre, M. A. 2020; Stutts, L. A., & Vermerris, W., 2020). Anthracnose disease in sorghum is brought on by the hemibiotrophic fungal pathogen *Colletotrichum sublineola* (Gutierrez-Martínez et al. 2016). The fungus produces red rot (stalk rot) and leaf spots (anthracnose). On both leaf surfaces, the disease presents as tiny, reddish dots. Red, purple, or brown edges surround the spot's white centre. Numerous tiny black dots resembling acervuli on the spot's white surface can be seen (Vikaspedia; Grain Sorghum | Texas Plant Disease Handbook).

Downy mildew

Peronosclerospora sorghi, a fungus-like organism that causes downy mildew on sorghum (Sorghum downy mildew - CABI.org; Vikaspedia; Wikipedia- *Peronosclerospora sorghi*, 2022). It is primarily a disease of the soil. Before infecting new plants, thick-walled oospores can remain viable in the soil for several years. Oospores can also be transferred from seed to seed. The fungus causes systemic downy mildew of sorghum. Through oospores or conidial infection, it enters the growth points of young plants. The leaves are coloured green or yellow as they unfold. On the lower surface of the leaves, there is much downy white growth made up of sporangiophores and sporangia (SORGHUM: MAJOR DISEASE).

Sorghum ergot

Claviceps africana is a fungus that causes sorghum ergot (Products, P. B. 2021) (Wikipedia contributors. 2022b). Through poor seed germination, the disease lowers yield. Sticky honeydew on seed heads makes harvesting difficult. Low nutritional value and sclerotes (ergots), which are fungal bodies, can affect grain quality (Heyman, G. 2021). Ergot does not infect paspalum or winter cereals like wheat and barley; only *sorghum* is affected. Even though ergot spores on sorghum seeds can live in honeydew, the fungicide thiram, frequently used on sorghum sowing seeds, will kill the spores (*Claviceps sorghi* (sorghum ergot). 2022, CABI Compendium).

The smut

The grain is replaced with a fungus structure packed with spores called a sori as the fungus spreads into the plant's ovaries. Because of this, smuts can be highly harmful to a sensitive sorghum crop when left unchecked (D, S, 2017).

Foliar blight

The fungus *Helminthosporium turcicum* is the cause of the disease known as leaf blight. (Rutkoski, J. 2019; Kiran, B. M., & Patil, P. 2019). Reddish-purple or tan dots that enlarge into larger lesions are the symptoms of the illness that appears on the sorghum plant's leaves, especially when wet. This fungus targets both young and mature plants (Kiran, B. M., & Patil, P. 2019). In Mexico, Brazil, India, Sudan, Nigeria, Niger, Kenya, and Ethiopia, sorghum leaf blight a critical disease that has a significant economic impact on forage and grain sorghum causes significant economic losses. (Anitha, K., Das et al., 2020; Pioneer, 2019).

Leaf rust and grey leaf spot

Sorghum can suffer economic harm from leaf rust and grey leaf spot, especially in humid settings. These foliar diseases are frequently linked to ratoon sorghum and late planting.

Sorghum can be infected with bacterial leaf spots or bacterial leaf stripes, but this typically only causes cosmetic leaf lesions and not financial losses (Pioneer. 2019). De Paula et al., (2022). Vikaspedia; Anitha, K., Das et al. 2020).

Leaf rust

Sorghum leaf rust is brought on by *Puccinia purpurea* (*Puccinia purpurea* -rust of grasses, sorghum, 2022. *Plantwise Plus Knowledge Bank*), which is widespread throughout the world's sorghum-growing regions. In some cool, humid regions of Central and South America, South-East Asia, and Southern India, rust epidemics nearly always occur each year, lowering pasture quality and grain yield (*Puccinia purpurea*, rust of grasses, sorghum. 2022, *CABI Compendium*, *CABI Compendium*)

Grey leaf spot

The pathogen *Gloeocercospora sorghi* is responsible for the illness known as the grey leaf spot of sorghum. It frequently occurs in hot, muggy years and overwinters on crop debris. In its early stages, the disease manifests as small, elongated, thin spots that eventually spread along the length of the leaf. Long elliptical necrotic lesions with dark edges that are straw-coloured in the centre are the typical symptoms of older plants. Up to 25% of the grain output may suffer damage due to the disease. Most Sorghum vulgare cultivars have susceptibility. Sorghum is susceptible to grey leaf spots nearly the entire growing season, with severity being highest in the latter half (Pioneer. 2019; *Grain Sorghum Disease*, Texas A&M AgriLife Research and Extension Center; De Paula, et al., 2022; Anitha, K., Das et al (2020).

Black leaf spot

Black leaf spot affects sorghum infrequently. Other leaf spot diseases of sorghum do exist, though, including zonate leaf spot of sorghum (Pioneer. 2019). ; Vikaspedia; Cota, L. V., 2010) which is brought on by the pathogen *Gloeocercospora sorghi* and sorghum anthracnose , which is brought on by the fungus *Colletotrichum graminicola* (Anitha, K., Das et al (2020).

There are other sorghum leaf spot diseases, however, including Sorghum anthracnose brought on by the fungus *Colletotrichum graminicola* and Zonate Leaf Spot of Sorghum caused by the pathogen *Gloeocercospora sorghi*.

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Chapter: 6**Awareness and Impact of Air Pollution on Biodiversity: Survey an Approach to Find Solution**

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Abstract: Ecological equilibrium is significantly influenced by biodiversity. It is an important genetic resource for people and aids in the survival of both people and other animals in the environment. The biodiversity is often threatened by air pollution. Every year, air pollution causes the early deaths of more than 7 million species. This study was carried out as a survey to learn about people's perceptions and awareness of environmental issues, such as how air pollution affects biodiversity. The survey was also conducted to see what strategies people are using or considering using to minimize air pollution and conserve biodiversity. In this study, participants were split into two groups: first, persons with a background in science or technology (students, academics, faculty members, etc.); second, the general public. To perform the research, a prepared question was sent to both groups using a Google form. Pie and bar charts were used to analyze the query. According to the survey, nearly 91% of respondents in both categories are aware of how air pollution affects biodiversity and are aware of its causes in a variety of settings. Almost 81% of people believe that companies are to blame for air pollution, and roughly 83% believe that each of us can do our part to lessen it. The influence of air pollution on biodiversity has been discussed in many contexts throughout this research. The study's findings suggest that more needs to be done to raise public understanding of how air pollution affects biodiversity and that it is essential for everyone to take steps to embrace habits that may lower air pollution. Such a poll and increased awareness of these environmental problems might be important first steps in figuring out how to save our planet.

Keyword: Air pollution, biodiversity, ecology, environment, humanity

Introduction

Thousands of chemicals are regularly used for industrial, agricultural, and household purposes around the world, and thousands more are developed each year (Maugh 1991). Most

of these compounds ultimately reach the atmosphere and may be dangerous to the health of plants, animals, and microbes since many of them are poisonous or radioactively active (Barker and Tingey, 1992). The gradual alteration in the composition of the atmosphere over the last century is caused by increased fossil fuel consumption. Ecotones, or natural ecoclimatic transitions, such as tropical tree border lines in rainforest-savanna boundaries, temperate timber lines in northern Finland, and the cloud forest-Paramo interface, are more vulnerable to climate change and have been shown to be indicators of extinction in the previous 30 years. Animals are exposed to warmer temperatures sooner as a consequence of climate change, which causes them to emerge from hibernation earlier (Prakash, et al. 2013). Different air pollutants, such as carbon monoxide (CO), sulphur dioxide (SO₂), nitrogen oxides (NO_x), ozone (O₃), heavy metals, and respirable particulate matter (PM_{2.5} and PM₁₀), have different chemical compositions, reaction characteristics, emission rates, times at which they disintegrate, and capacities for long- and short-distance diffusion (Kampa and Castanas, 2008). Studies have shown that globally dispersed air pollution is having an impact on biota and ecosystems (Barker and Tingey, 1992). A broad range of contaminants, such as dust, smoke, smells, fumes, mists, and gases, are included in air pollution. Industries, factories, energy-processing facilities, vehicles, aircraft, municipal waste incinerators, and agricultural activities all contribute to the discharge of these air pollutants. These pollutants are produced by chemicals, metal, plastic, pulp, and paper industries (Moser et al., 1992). Lake acidification, eutrophication in estuaries and coastal waterways, and mercury bioaccumulation in aquatic food webs are all caused by or exacerbated by air pollution. While the impacts of air pollution on biogeochemical cycling in terrestrial ecosystems are well established, it is less clear how air pollution affects most species and how it interacts with other stressors. Yet, there is compelling evidence that nitrogen deposition has an impact on plants in bogs, alpine regions, and grasslands, as well as on forest mycorrhizae (Lovett, et al. 2009). Inorganic gases created by the burning of fossil fuels, such as sulphur dioxide and nitrogen oxides, as well as the acids that result from them in "acid rain," have garnered the majority of attention in the impacts of air pollution on plants (Cape, 2003). Exposure to air pollution is linked to a number of serious negative health effects (Brook et al., 2010). Fine particulate matter (PM_{2.5}) exposure has been linked to a variety of health issues, including cardiovascular and respiratory issues (Mahsin et al., 2022). PM 2.5 is made up of a variety of substances, including metals, black carbon, and polycyclic aromatic hydrocarbons, all of which have varying potential health impacts (Mukherjee and Agrawal 2018). Inhaled toxic metals, the main damaging component of PM_{2.5}, produce oxidative stress, which results in

endothelial and cardiac dysfunction. (Miller et al. 2012) Asia has recently seen fast economic expansion, environmental degradation, and an epidemic-level rise in allergy illnesses. The levels of air pollution in many Asian nations, particularly in China and India, are far greater than in Western nations. Additionally, indoor pollutants from chemicals and tobacco as well as industrial, traffic-related, and household biomass combustion are significant sources of air pollutants, adding to the burden of respiratory allergies (Pawankar et al. 2020). Many epidemiological studies have demonstrated that short-term exposure (Shang et al., 2013) long-term exposure (Kim et al. 2017) to atmospheric pollutants may negatively affect human health (Xia et al. 2019). Epidemiology studies indicated that air pollution has been associated with adverse neurological effects in human (Jia et al., 2018). Also, the results of research conducted in China show that exposure to air pollution may have a role in sadness and decreased levels of enjoyment while relaxing (Zhang et al, 2017). This study was conducted to examine the general understanding of individuals from various groups on air pollution and its impact on biodiversity. This survey also aimed to raise awareness among individuals of all ages and demographics so that they might each take personal responsibility for helping to safeguard the environment by decreasing pollution. Also, they will learn about how air pollution affects biodiversity. These survey studies may raise awareness of these environmental challenges, and they can also be useful in identifying information gaps, examining policy concerns, and directing future research so that we can develop innovative methods to save our planet from pollution and the extinction of species.

Methods and Materials

Google forms were used to carry out our survey and <https://forms.gle/A2Fid4wCeH9SZvHG9> is the survey's URL. The survey was first divided into categories for those working in scientific/technology fields and those working in fields other than science and technology in order to analyse the viewpoint on the problem and individual knowledge in depth. Individuals were also divided into age groups (0-17; 18-24; 25-34; 35-44; 45-54; 55-64; 65-74; 75 or more). In order to relate the survey to the backgrounds of those who participated in it, the occupation group (Professor, Research Scholar, Student, Homemaker, Non-Working, Others, etc.) was also stated in the research of the association between age group and profession. According to the questions, the poll was roughly split into three categories. Biodiversity, air pollution, and how air pollution affects biodiversity. These three parts include questions on awareness, knowledge, sources, causes,

effects, and solutions to problems. Each of these questions relates to the respective segment as mentioned in Table 1.

Table 1: Questionnaire prepared for the survey

S.no.	Sections	Name of Section	Questions
1	Section 1	Biodiversity	Q1) Which of these animals do you think are endangered? Q2) Which of these plants do you think are endangered? Q3) What do you think is the main cause of extinction? Q4) Do you think there is enough awareness about endangered species? Q5) How concerned are you about animal/plant extinction?
2	Section 2	Air Pollution	Q1) How much would you rate overall air quality in your city now compared to last year. Q2) What do you think is the main cause of Air Pollution. Q3) In which of the following ways are you affected by the Air pollution? Q4) Do you burn coal/woods in winters to keep yourself warm? Q5) Do you use air conditioners in summers for a very long period of time?
3	Section 3	Air pollution affecting biodiversity	Q1) Do you think air pollution affects biodiversity. Q2) If air pollution is affecting biodiversity so major problems occur with. Q3) As per your view, the reason behind the biodiversity linked with air pollution could be. Q4) Do you think an individual can make some effort to reduce air pollution. Q5) For your answer to above question give reason.

Result

Categories of the people participating in survey

The categories planned to collect the information of participating people was age, occupation, and specific category of science/technology field or General other than science and technology. These categories help to understand the learning and knowledge of the participant on the subject. This survey was the matter of concern for every individual so it was equally important that all the people should participate and, we can segregate the result as per their knowledge and learning. Total of 100 people participated in the survey among them the 77% of students were of age 18-24, 7% were of age 0-17 and rest in other age groups such as 25-34, 35-44 and 45-54 were 9%, 4%, and 3% respectively.

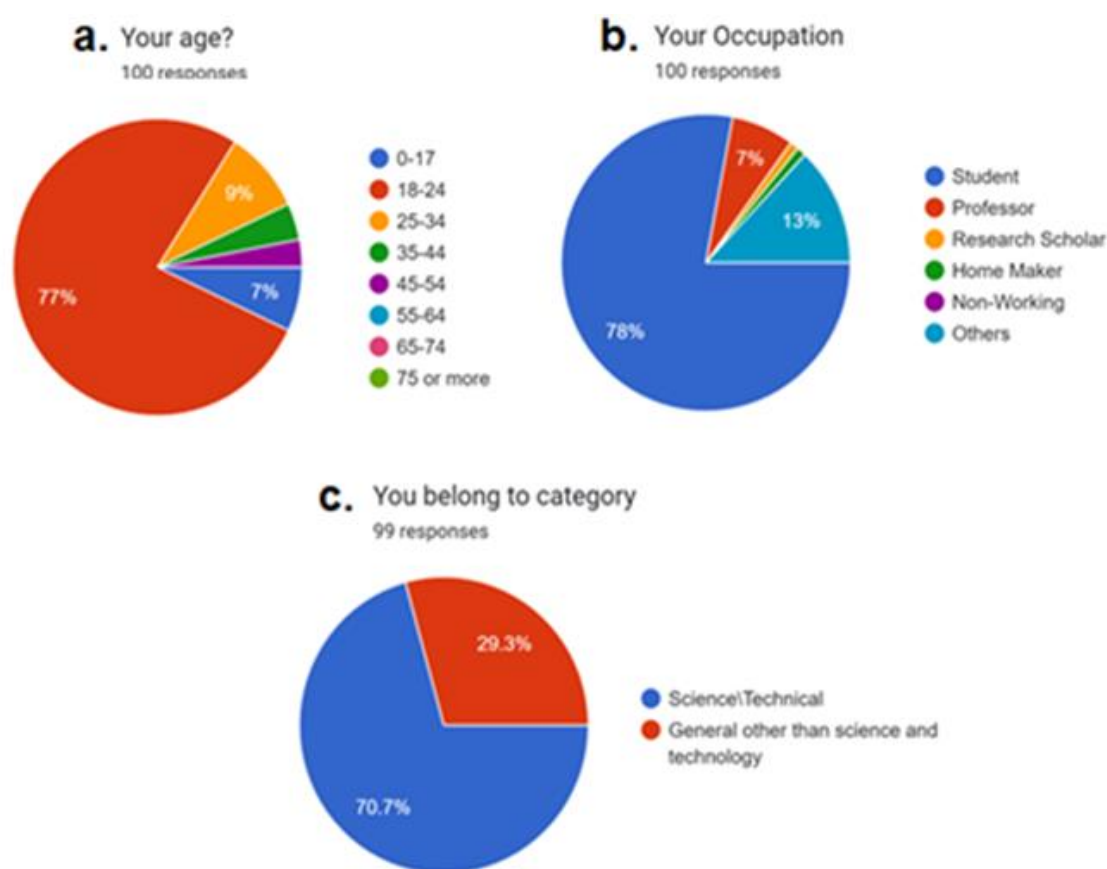


Figure 1: Information and details about the participants (a) Age (b) Occupation (c) Category

The maximum people participated in survey was students almost 78% and then other categories 7%. These results show that the maximum student's participation in such survey as the students are more aware of the subject. Among the participants 70.7% of people were from science background and rest 29.3% people were from non-scientific background.

Awareness and cause of the endangered and extinct biodiversity

The awareness study was carried out for the extinction of biodiversity and endangered species. The questions were designed in such a way so that every participant can relate to the

subject, easily understand the question, and respond honestly and without hesitation. This will help to gather accurate data for the survey.

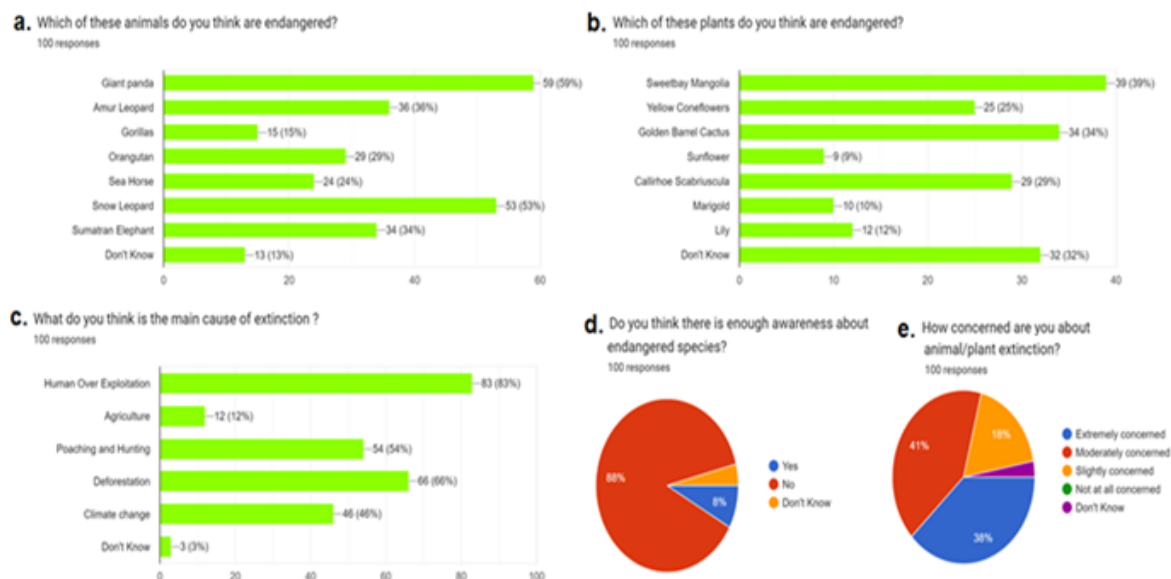


Figure 2: Survey outcome of the endangered species and Biodiversity (a) Endangered animals (b) Endangered plants (c) Main cause of extinction (d) Awareness about endangered species (e) How concerned are you about animal/plant extinction

These results show that 59% participant vote for *Ailuropoda melanoleuca* (Giant Panda), 53% for *Panthera uncia* (Snow Leopard), 36% for *Panthera pardus orientalis* (Amur Leopard), 34% for *Elephas maximus sumatrensis* (Sumatran Elephant), 29% for *Pongo* (Orangutan), 24% for *Hippocampus* (Sea Horse), 15% for *Gorilla* (Gorilla) as the most endangered animal species and almost 13% participant don't know anything about it. 39% participant thinks that *Magnolia virginiana* (Sweet Bay Magnolia), 34% *Echinocactus grusonii* (Golden Barrel Cactus), 29% *Callirhoe scabriuscula* (Texas Poppy Mallow), 25% *Echinacea paradoxa* (Yellow Coneflowers), 12% *Lilium* (Lily), 10% *Tagetes* (Marigold), and 9% *Helianthus* (Sunflower) are the most endangered plant species and almost 32% participant don't know which gives us the idea that participants are more aware about animal extinction compared to that of plant. 83% of participants think that human over exploitation, 66% deforestation, 54% poaching and hunting, 48% climate change, and 12% people think agriculture is the main cause of extinction and 3.9% don't know the reason for extinction. 88% of participants think that there's not enough awareness about endangered species, 8% thinks that there's enough awareness, and about 4% have no idea about it. 41% participant are moderately concerned, 38% extremely concerned, 18% slightly concerned about

animal/plant extinction, and 3% participant are not sure about the issue and from this we get a idea that majority of participant has a broad knowledge of the issue and all of them are concerned about animal\plant extinction.

Awareness and causes about air pollution

The awareness and causes of air pollution was studied by putting questions such as how people are using things that are causing air pollution and that is affecting environment and human health. The survey helped in understanding; what people think about the main cause of air pollution.

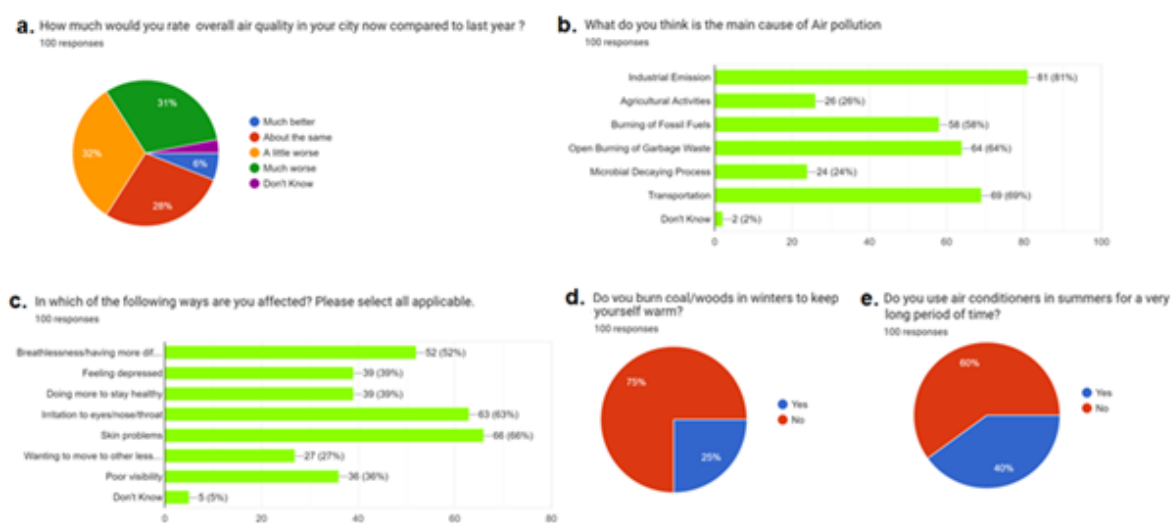


Figure 3: Survey outcome about the Air Pollution (a.) Air quality (b.) Main cause (c.) Ways in which air pollution affects (d.) People who burn coal/woods in winter (e.) Long time use of Air Conditioner

The result shows that 32% of participants think that the air quality in their city is little worse, 31% think much worse 28% about the same 6% much better as compared to last year and almost 3% participant don't know. 81% participant thinks that industrial emission 69% transportation 64% open burning of garbage waste 58% burning of fossil fuels 26% agricultural activities 24% microbial decaying process is the main cause of air pollution and about 2% participant don't know. Among the participants 66% are affected by skin problem 63% irritation to eyes/nose/throat 52% having more difficulty in breathing/breathlessness 39% feel depressed 39% needs to do more to stay healthy 36% poor visibility 27% wants to move to a less polluted place as they suffer from air pollution and about 5% participant don't know how they were affected by air pollution and what they want as the data shows participants are well aware about their own city air quality and the main cause of air pollution causing it and how they were being affected by bad air quality and air pollution.

Almost 75% participant burns coal/woods and 25% don't to keep themselves warm in winters and about 60% participant uses air conditioner for a long time and 40% don't in summers as the data shows most of the participant contribute to air pollution unknowingly by performing these activities.

Awareness and causes about air pollution affecting biodiversity

In this last section impact of air pollution on biodiversity was studied also about what people think that how air pollution is affecting biodiversity and if it is then what are the main problems that occur with it. The questions were also planned to study the weather an individual can contribute alone for the betterment of environment. The main reason behind the biodiversity linked with air pollution was to know the knowledge and perspective of the people on the current topic and how they can contribute in solving the problem.

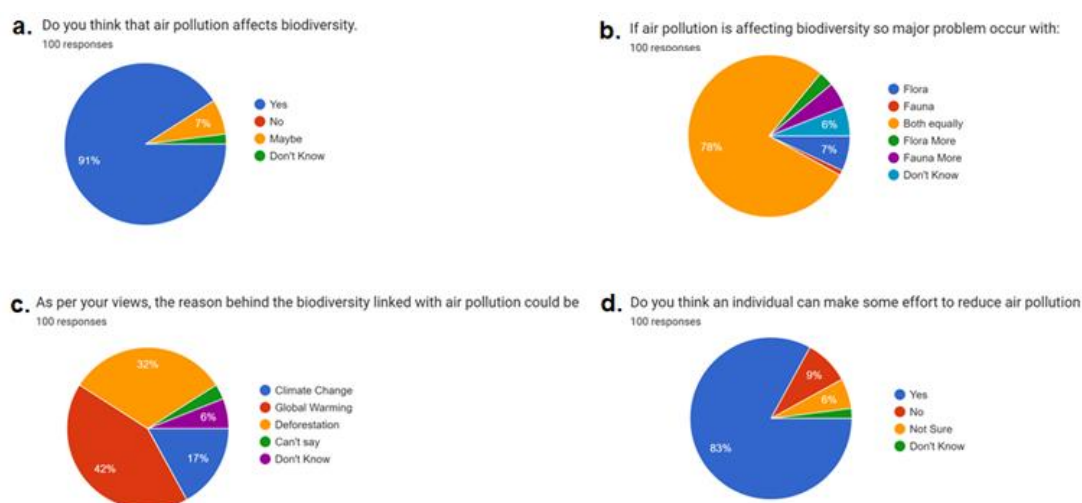


Figure 4: Information about the Air Pollution affecting Biodiversity (a.) Does air pollution affect biodiversity (b.) major problem if air pollution is affecting biodiversity (c.) the reason behind the biodiversity linked with air pollution (d.) an individual can make some efforts to reduce air pollution?

The results show that 91% participant think that air pollution affects the biodiversity whereas 7% don't think that air pollution affects the biodiversity and about 2% Don't know. Among them 78% thinks that the major problem occurs with Flora and Fauna equally 7% thinks it occurs with Flora 5% think it occurs with Fauna more than Flora 3% think it occurs with Flora more than Fauna 1% occurs with Fauna and about 7% participant Don't know. And from that about 42% participant think that Global Warming 32% Deforestation 17% Climate change could be the reason behind the biodiversity linked with air pollution among

them almost 3% participant can't say what is the reason behind the biodiversity linked with air pollution will and about 6% Don't know what the reason behind biodiversity is linked with air pollution is. Among these 83% participant think that an individual can make efforts to reduce air pollution 9% doesn't think so and 6% are not sure if an individual can make efforts of their own to reduce air pollution and about 2% participant Don't know.

Conclusion

In the survey people of both the categories science/technical and general other than science and technological has given their views on their beliefs, knowledge and practices till date. The survey conclude that the people belonging to either category is equally concern about our environment as much as the other and has given solution to tackle the increasing air pollution affecting the biodiversity and ultimately our whole environment they suggested to use bicycle for short distance travel which keep the environment safe as well as our health will also be in right place by doing exercise while using bicycle to travel around and E-Vehicles for transportation which will decrease the carbon release in our environment and will benefit the environment as vehicles on daily basis produce a large amount of air pollution affecting our surrounding and affecting our health little by little which is not evident instantly but shows symptoms in a long run and decreasing the life span of every living thing not just only human beings.

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Chapter: 7

**Screening of some antipathogenic botanicals in vitro and field conditions against
Colletotrichum falcatum causing red rot of sugarcane**

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Abstract: The present study evaluated with the antipathogenic perspectives in vitro impact of (Aqueous and Ethanolic) antifungal plant (foliar) extracts (0, 5, 10, 15 conc. %) of eight indigenous plants *Psidium guajava*, *Sonchus oleraceus*, *Chenopodium album*, *Cassia tora*, *Achyranthus aspera*, *Euphorbia hirta*, *Euphorbia thymifolia* and *Phyllanthus niruri* against *Colletotrichum falcatum*, the pathogen of red rot disease of sugarcane. Pour plate method was used to determine the effect of different leaf extracts (Aqueous and Ethanolic). On mycelial radial growth of *C. falcatum*. The aqueous and ethanolic leaf extracts of all the botanical species have significant effect on tested pathogen. The gradient of efficacy of the leaf extracts of 8 types of leaf extracts tested for antifungal efficacy against *C. falcatum* was: *Psidium guajava* > *Cassia tora* > *Phyllanthus niruri* > *Euphorbia hirta* > *Achyranthus aspera* > *Sonchus oleraceus* > *Euphorbia thymifolia* > *Chenopodium album*. Ethanolic extracts proved more effective as compared to aqueous leaf extract regardless of the extract and its concentrations used in this study. Maximum inhibition 86.44% by ethanolic and 69.4% by 15% aqueous extract was found as a result of the *Psidium guajava* leaf extract followed by *Cassia tora* and *Phyllanthus niruri*. The poorest efficacy was noted with *Chenopodium album* and *Sonchus oleraceus* irrespective of the type of the extractant used. In another (field) experiment conducted during 2018-2019 (spring planting) with four red rot disease susceptible (CoPk 05191, CoO238, CoO118 and CoJ64) sugarcane varieties to evaluate the impact of leaf ethanolic extracts (15 % conc) of eight antipathogenic botanicals sprayed at 180 DAP for the study of leaf nutrient contents (NPK %) at 240, 300 DAP and at harvest together with final cane yield at harvest only. It was observed that highest cane yield 16.44 % more than the control (sprayed with water only) T₁ was noted with *Psidium guajava* (PS), T₂ which was statistically equal to *Phyllanthus niruri* (PN), T₉ followed by *Cassia tora* (CT), T₅

which was associated with high leaf phosphorus and potassium percentages found at almost all stages studied. The lowest cane yield was noted with *Chemopodium album* (CA), T₄ leaf extract as compared to control (T₁). Variety CoO238 responded most followed by CoO118 in final cane yield. CoJ64 was poorest in performance. There was an accumulation of nitrogen content in the untreated plants (T₁) at all growth stages studied. The magnitude of accumulation varied according to the degree of resistance of cane varieties.

Keywords: Antipathogenic leaf extracts, in vitro, field conditions, red rot of sugarcane.

(A) In Vitro:

Introduction

The crux of the sugarcane problem is red rot disease of sugarcane caused by fungus *Colletotrichum falcatum* resulting in considerable damage and low yield of crop. Generally fungicides are routinely used for management of control of this disease but it has resulted in the development of fungal resistance to the chemicals and environmental hazards. Although many strategies were developed to tackle the disease but still there is no strong alternative of chemical pesticides. It is an uphill task to subside and manage red rot disease in the field. Indian Sugar Mills Association (ISMA), which represents private sugar mills of the country has pegged India's 2021-2022 sugar production at 310 lakh tonnes after considering diversion of 34 lakhs tonnes for production of ethanol. It has estimated an increase of 117 crore liters in ethanol production, which is 35% more than 333 crore liter expected to be produced in 2020-2021. After accounting for the reduction in sugar production due to diversion of cane juice and B-molasses to ethanol, ISMA estimates sugar production in 2020-21 at around 310 lakh tons of sugar. This will be similar to the current year's sugar production, Bhosale (2021). In 2021-2022, Uttar Pradesh largest producer of sugarcane in India is estimated to have sugarcane area at 23.12 lakh hectares as against 23.07 hectare in 2020-2021 which is more or less similar to previous year. ISMA is expecting a marginal increase as yield as well sugar recovery and thus the estimated sugar production, without considering diversion for production of ethanol in 2021-22 is expected to be around 119.27 lakh tonnes, Bhosale (2021).

Shamli district in West UP region of India has topped by producing over 1004 quintal per hectare of sugarcane followed by Muzzaffarnagar 923.20 quintal per hectare. But crop production sometimes deteriorated due to various stresses, pests and diseases. The worldwide loss in cane yield and sugar recovery is approximately 5-10 % per annum (Viswanathan &

Samiyappan, 1999). Fungi are responsible for numerous diseases of plants resulting in substantial loss in crops yield. The red rot disease of sugarcane is caused by the fungal pathogen *Colletotrichum falcatum* Went. It is one of the oldest diseases of sugarcane and is of great economic importance and a serious threat to the sugar industry particularly in subtropical countries (Viswanathan & Samiyappan, 1999; Guerber & Correll, 2001; Alexander & Viswanathan, 2002; Armstrong-Choa & Banniza, 2006 and Tariq et al. 2017). It is caused by prolonged storage or exposure to high relative humidity, environmental pollution and high temperature. In particular, red rot occurs at pH values of 4.2 to 4.5, sulphur dioxide converts to sulfurous and which forms hydrogen peroxide. The peroxide combines on the residual tannins in the leather to oxidise proteins creating ammonium sulphate and ammonium bisulphate. In India, the estimated loss in crop production due to fungal diseases is 18-31 % Jayashree et al. (2010). It can reduce cane weight by up to 29% and loss in sugar recovery by 31% Hussnain and Afghan (2006). As a result the quantity of molasses increases (Sehtiya et al. 1993). It is aptly called the “Cancer of Sugarcane” Khan et al., 2011 and Sharma and Tamta, 2015. Its inoculum is disseminated by wind rain, heavy dews or irrigation water. Infected cane setts can readily spread or cause secondary infections whole crop debris or stubble may also provide inoculum to infect a new crop (Raid, 2012).

The recent research interest with economical sustainable perspectives in the use of some medicinal plants parts for control of some plant and animal diseases can be related to presence of secondary metabolites which have antimicrobial properties such as cyanogenic glycosides, tannins, polyphenols etc. in them (Iwu, 2000; Costa et al. 2000; Duru et al. 2003; Nasir et al. 2014; Coley et al. 2003; Shirnwari 2010; Kumar & Yadav 2007; Nagarajan 1993; Spencer et al. 1957; Prabakaran and Prince 2011; Jagtap et al. 2009; Mandali et al. 2009; Nwachukwu & Umechuruba 2009; Javaid & Rehman 2001; John et al. 2016; Suleiman 2011; Akhtar & Azam 2014). Non effectiveness development of resistance and phytotoxicity of synthetic fungicides for the control of plant diseases call for the search for natural products as alternatives. Once the infection of *Colletotrichum falcatum* occurs is very difficult to control the red rot disease in field. Since the general use of chemical fungicides has resulted in the development of fungal resistance to the chemicals and environmental hazards it becomes imperative to search new strategies and antifungal herbal potentials to combat the disease (Spencer et al. 1957; Cowan, 1999; Kakonen et al. 1999; Abad et al. 2007; Prabakar & Prince 2011; Bhardwaj & Sahu, 2014; Patel et al. 2015).

To evaluate some more sustained effective possibilities on control of this serious disease present in vitro investigation was conducted on eight medicinal plants extracts (aqueous and ethanolic) in 0, 5, 10 and 15% concentration with aim to know efficacy of antifungal activity against the test fungus *C. falcatum*.

Material and methods

Fresh leaves of *Psidium guajava*, *Sonchus oleraceus*, *Chenopodium album*, *Cassia tora*, *Achyranthus aspera*, *Euphorbia hirta*, *Euphorbia thymifolia* and *Phyllanthus niruri* having active constituents as follows

1. *Psidium guajava*: Serquiterpenes, fatty acids, caryophyllene α – copaene, Cis-muurolo, α – cardinol, α – bisabolol, cyclosativene, cubenol, guajanoic acid, uvaol, oleonotic acid, and ursolic acid.
2. *Sonchus oleraceus*: Sesquiterpenes, luteolin, apigenin, kaempiferol, quercitin, flavonoid glucosides, luteolin – 7-O-D-glucoside, apigetrin, astragalin, isoquercitrin.
3. *Chenopodium album*: Trypsin inhibitor activity, phenols, tannins, saponnins, phytic acid, phytate phosphorous, alkaloids, flavonoids, oxalates, oils, proteins.
4. *Cassia tora*: Anthraquinone glycosides, flavanoids, rhein, emodine, physion, chrysophenol, obtusin.
5. *Achyranthus aspera*: Triterpenoid saponins, oleonolic acid, glycone, ecdysterone.
6. *Euphorbia hirta*: alkanes, triterpenes, phytosterols, tannins, polyphenols and falvonoids, triterpenoids, coumarins, lignin.
7. *Euphorbia thymifolia* alkanes, triterpenes, phytosterols, tannins, polyphenols and flavonoids.
8. *Phyllanthus niruri*: Lignan, tannains, coumarins, terpenes, flavonoids, alkaloids, saponins, and phenylpropanoids.

were collected and washed properly under a tap water. The leaves were than air dried separately on a laboratory bench for fourteen days and ground into powder using an electric grinder. To obtain the three concentrations (5, 10 and 15%) assayed in this study. 5, 10 and 15 grams in 100 ml of 70% ethanol (ethanolic extraction) and 100 ml of sterile distilled water (aqueous extraction) in different 250 ml conical flasks. Each of the conical flasks was covered with aluminum foil and allowed to stand for 48 hrs on the laboratory bench. The extracts concentrations were sterilized and filtered into labeled sterile bottles with cork and then stored at 10°C prior to use.

The pour plate method as described by Olahan and Amadi (2006) was used to determine the efficacy of each of the sterile ethanolic and aqueous leaf extracts on the radial growth of *C. falcatum* on Potato Dextrose Agar (PDA) plates. Four equal sectors were created on the cover of each of sterile disposable petridishes by drawing two perpendicular lines running through its centre. About 2 ml of 70% ethanol was introduced separately into sterile disposable petridishes with a new 5 ml hypodermic syringe and labeled as control E (controls for the ethanolic leaf extracts). The same value of sterile distilled water was also introduced separately into another sterile petridishes with a new 5 ml hypodermic syringe and labeled control aqueous (control for the aqueous leaf extracts). The 2 ml of each type of leaf extracts to be assayed for each of the concentrations were separately inoculated into different sterile disposable petridishes for in- vitro antifungal efficacy trials.

All inoculated petridishes were arranged in a Completely Randomized Design (CRD) on the work bench and served with 15 ml of molten PDA culture medium amended with 1 ml of streptomycin BP under aseptic condition. The petridishes were labeled approximately and swirled gently to homogenize its contents and then allowed to solidify on the bench.

Mycelial plugs (4 mm in diameter each) were picked from advancing edges of 5 –day old pure culture of *C. falcatum*. A plug of *C. falcatum* was placed in an inverted position at the centre of each disposable petridishes containing PDA amended with different concentrations of the extracts (treatment plates) as well as control plates using a sterile inoculating needle. All the inoculated plates were incubated at 25°C for 6 days. The diameter of the mycelium on each plate was measured daily with a 30 cm transparent plastic ruler and recorded. The mycelial extension of the pathogen was determined using the formula

$$D_{mm} = \frac{d_x + d_y}{2}$$

Where D = Diameter of the mycelium

D_x = Diameter along the horizontal line

D_y = Diameter along the vertical line on each of the PDA plates

The percentage growth inhibition of each of the treatments and concentrations was determined using the formula cited by Okigbo and Nmeko (2005), i.e.

$$\text{Growth inhibition by a leaf extract (\%)} = \frac{D_c - D_t}{D_c} \times 100$$

Where Dc = diameter of the test microorganism on the control plate

Dt = diameter of the test microorganism on the experimental plate.

Data for the percentage growth inhibition of the leaf extracts were subjected to analysis of variance (ANOVA) and the treatment mean percentage growth inhibition values were separated by Duncan's Multiple Range Test (DMRT) at $P < 0.05$ using software IBM SPSS statistics Package Version 25.

Table 1: Mean and SE of clear zone diameter of *Colletotrichum falcatum* as influenced by different plant foliar extracts

Con c %	<i>Psidium guajava</i>		<i>Sonchus oleraceus</i>		<i>Chenopodium album</i>		<i>Cassia tora</i>	
	Aqueous	Ethanollic	Aqueous	Ethanollic	Aqueous	Ethanollic	Aqueous	Ethanollic
0	5.9±0.35	5.9±0.25	5.9±0.36	5.8±0.29	5.9±0.38	5.9±0.27	5.9±0.26	5.9±0.10
5	3.4±0.42	2.1±0.34	4.8±0.44	3.3±0.33	4.2±0.24	3.7±0.35	3.3±0.38	2.0±0.20
10	2.8±0.44	1.1±0.33	3.2±0.42	3.0±0.34	3.8±0.28	3.2±0.37	2.9±0.33	1.1±0.10
15	1.8±0.36	0.8±0.29	3.0±0.35	2.9±0.25	3.7±0.17	3.0±0.36	2.8±0.22	0.9±0.10

*values are significantly different at $p < 0.05$, DMRT

Table 2: Mean and SE of clear zone diameter of *Colletotrichum falcatum* as influenced by different plant foliar extracts

Con c %	<i>Achyranthus aspera</i>		<i>Euphorbia hirta</i>		<i>Euphorbia thymifolia</i>		<i>Phyllanthus niruri</i>	
	Aqueous	Ethanollic	Aqueous	Ethanollic	Aqueous	Ethanollic	Aqueous	Ethanollic
0	5.9±0.20	5.9±0.33	5.9±0.21	5.8±0.10	5.9±0.22	5.9±0.26	5.9±0.25	5.9±0.22
5	4.1±0.15	3.9±0.21	4.7±0.24	3.7±0.30	4.3±0.12	3.7±0.22	3.1±0.18	2.9±0.28
10	3.3±0.22	3.1±0.11	3.8±0.20	2.9±0.28	3.9±0.14	2.4±0.15	2.9±0.22	2.0±0.10
15	2.9±0.21	2.7±0.10	3.1±0.22	2.4±0.22	3.3±0.20	2.3±0.18	1.7±0.18	1.8±0.20

*values are significantly different at $p < 0.05$, DMRT

Results and Discussion

It was observed that the mycelial diameter of *C. falcatum* increased slowly but progressively throughout the incubation period. There were reduction in the mycelia growth of *C. falcatum* in the treated petridishes compared to mycelial diameters in the control plates and these applied to both aqueous and ethanolic leaf extracts (Table 1 and 2). The ethanolic leaf extracts were more active and strong in retarding the mycelial radial growth of *C. falcatum* in vitro as compared to aqueous extract across all the concentrations studied and this impact

increased within the period of incubation of *C. falcatum* with 5, 10 and 15 % concentration of leaf extract was noted with ethanolic of *Psidium guajava* 64.4, 80.3 and 86.44 % respectively as compared to control (Table 1) at $P < 0.001$. This was closely followed by the effect of leaf ethanolic extract of *Cassia tora* (66.1, 80.3 and 84.7 % respectively) with the similar concentrations as compared to control (Table 1). The antifungal potential of *Psidium guajava* Linn. leaves essential oil and its major compounds beta caryophyllene and caryophyllene oxide were tested by Jassal et al. (2021) against *Fusarium moniliforme*, *Rhizactonia solani* and *Helminthosporium oryzae* by using poison food technique. Guava leaves essential oil was most effective against *Fusarium mumliiforme* having ED50 value of 280 $\mu\text{g} / \text{ml}$. Caryophyllene was most effective against *Rhizactona solani* and helminthosporium having ED50 values of 450 and 510 $\mu\text{g} / \text{ml}$ respectively. Also caryophyllene Selin – 7 (II)-en-4 α – ol for the leaf oil of *Psidium guajava* radical scavenging acyintues were assessed both reduced DPPH, diphenylpicryl hydrazyl and deoxyribose degradation assays with $^{1C}50$ values of 3.59 and 12.64 $\mu\text{g} / \text{ml}$. The anti-inflammatory activity was explained via virtual docking of the major identified compounds to the main sites in the 5-lox crystal structure (El Ahmady et al. 2013). The leaves chemical constituents of *Psidium guajava* have also been worked out by Shaheena et al. (2019).

Similarly Patel and Shah (2019) noted antimicrobial activity of petroleum ether and ethanolic extracts of *Cassia tora* seeds and leaves. Anthraquinones were found in ethanolic extract of leaves and seeds. Each of the extract was screened for antimicrobial against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. Ethanolic extract of leaves showed significant anti-microbial activity. The MIC of ethanolic extract of leaves was found to be 100 mg/ml, 75 mg/ml and 50 mg/ml against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* respectively. Therefore antifungal activity of *Cassia tora* leaf extracts in our study is quite understandable. The lowest response of mycelial inhibition on *C. fatcatum* due to different (5, 10 and 10 % leaf ethanolic extracts etc) concentrations was noted with ethanolic extracts of *Chenopodium album* (37.2, 45.7 and 49.9% respectively) Naqvi et al. (2019) also found all concentrations (1 to 5%) of *Chenopodium spp* leaf extract against *Furasium oxysporum* sp. lycopersici reduced mass 14-45 %. The moderate antifungal activity in our study of leaf extracts of *Euphorbia thymifolia* also by (Mali & Panchal, 2013) *Euphorbia hirta* also by (Bhaskar Rao et al. 2010; *Sonchus oleraces* also by Banaras et al. 2020; *Achyranthus aspera* also by Singh et al. 2018 can also be correlated and found significantly effective by all these workers. Hence, these plants can be used to discover

bioactive natural products (various secondary metabolites) that may serve as leads in the development of new phyto-pharmaceuticals research activities.

(B) Field Conditions:

Introduction

The red rot disease of sugarcane caused by *Colletotrichum falcatum* went, fungi affecting both in yield and juice quality losses to both cane growers and sugar factories (Gill et al. 2012). A large number of high yielding and promising varieties have been withheld or discontinued by farmers from time to time in different areas due to their high susceptibility to this disease. Most of the high yielding sugarcane varieties are succumbed to red rot disease with further more breakdown in the resistant sugarcane genotypes due to its ongoing evolution (Kaur et al. 2014; Sharma & Tamta 2015). The red rot pathogen being a facultative parasite, keeps on mutating in nature resulting information of new races responsible for the breakdown of resistant varieties under field conditions (Kaur et al. 2014). Hence, all the time under the risk of emergence of this disease in susceptible sugarcane varieties due to dormant infections of the fungus in seed canes under field conditions (Viswanathan & Samiyappan 2002). Ecofriendly and sustainable alternative approach to manage disease is one of the most important aspect as we know that non effectiveness, development of resistance and phytotoxicity of synthetic fungicides for the control of diseases call for the search of natural products as alternatives. Hence, effort to expand the spectrum of antipathogenic agents from natural resonances merits intensive study based on plant extracts, had also been reported to suppress *C. falcatum*. It has been reported that ginger, onion and garlic extracts can inhibit mycelia development of *C. falcatum*. Applications of essential oils such as menthe oil, patchouli oil, peppermint oil and palm oil can also mitigate. *C. falcatum* infections (Bhardwaj & Sahu 2014). According to Imtiaz et al 2007, *Datura metal* and *Curcuma domestica* leaf extracts can inhibit both mycelia and conidial growth of the red rot pathogens. Similarly tobacco and dhup smoke (incense) are thought to inhibit the red rot conidial germination. The present study was conducted to assess the impact of eight different ethanolic antifungal leaf extracts (15% conc.) sprays applied at 180 DAP on four (CoPk 05191, CoO238, CoO118 and CoJ64) selected red rot susceptible sugarcane varieties under local conditions with respect to leaf nutrient (NPK) content and cane yield.

Material and methods

A field experiment was carried out (2018-2019 spring planting) at agricultural farm of G.F. College, Shahjahanpur, U.P., India with four red rot disease susceptible, CoPk 05191 (V₁), Co0238 (V₂), Co0118 (V₂) and CoJ64 (V₄) sugarcane varieties to assess the impact of different (*Psidium guajava*, PG, T₂; *Sonchus oleraceus*, SO, T₃; *Chenopodium album*, CA, T₄; *Cassia tora*, CT, T₅; *Achyranthus aspera*, AA, T₆; *Euphorbia hirta*, (EH), T₇; *Euphorbia thymifolia*, ET, T₈ and *Phyllanthus niruri*, PN, T₉) ethanolic antifungal leaf extracts (15 % conc) sprays applied at 180 DAP with a control (T₁) sprayed with water only for the study of leaf nutrient (NPK) contents at 240, 300 DAP and at harvest as well as final cane yield at harvest only. Single budded setts were sown in 5 x 4 sqm. beds, randomly each treatment replicated thrice in a factorial randomized block design. Soil was sandy loam, pH 7.1, organic carbon 0.4 %, available N 50, P 80 and K 100 kg/ha with nil calcium carbonate content. A basal dose of 150 kg/ N, 60 kg P and 100 kg K/ha was given in each bed before planting. The doses of nitrogen was splitted in 1/3 + 1/3 + 1/3 and first was given at the time of planting and rest two before monsoon as top dressing. All seed materials (setts) firstly dipped in 1 % solution of propiconazole for sterilization. Sampling for leaf nutrient (NPK) contents was done at 240, 300 DAP and at harvest stages and third leaf was selected for the purpose. Cane yield was noted at harvest only. The seed rate was (90 X 45 cm) row to plant spacing's. The general agronomic practices for the sugarcane crop was employed.

Leaf nutrient (NPK) contents

Leaf analysis is now well established as a tool for assessing the nutritional status of a particular crop plant (Lundegardh, 1951). Leaf analysis shows the relationship between nutrient contents, vegetative growth, index value of nutrients at various stages of the crop and requirement of the crop. However at other side, the soil nutrients not always shows correlation with the plant growth which may be because of various factors such as area of absorbing surface, antagonism, synergism, soil fixation etc. Dried leaves were taken, finely powdered and passed through a 72 mesh screen.

The powder was stored in polythene bags, labelled and kept for analyses. Dried leaves powder were taken from the plants sampled at the stages mentioned earlier, for nutrient analyses.

The following leaf nutrients at selected (240, 300 DAP and at harvest) stages were estimated on the basis of percent dry weight.

1. Leaf nitrogen content (%)
2. Leaf phosphorus content (%)

3. Leaf potassium content (%)

Digestion of plant samples

100 mg dried powder of leaf was transferred to flask (50ml) which contains 2ml sulphuric acid. In temperature controlled assembly, flask was exposing to heat for 2 hrs for the nitrate reduction which is present in plant material. After heating, the contents were turned black in flask. 30% hydrogen peroxide (0.5ml) was added and the mixed solution was heated till the changes in colour of solution occurs (black to light yellow) Now the flask was left for 30 min for cooling, then 3-4 drops of hydrogen peroxide (30%) was added and again heated for 15 minutes. The process was repeated. The final material was transferred to flask and final volume was maintained up to 100ml.

Estimation of leaf nitrogen

The method of Lindner (1944) was followed for estimation of nitrogen. Following this method, 10 ml of aliquot of digested material was taken in volumetric flask (50 mL). 2 ml of 2.5 N sodium hydroxide was for neutralize the excess of acid and 1 ml of 10% sodium silicate the check the turbidity. The volume was adjusted with DDW. 5 mL aliquot of the solution alongwith 0.5 mL Nessler's reagent was added in test tube and final volume was makeup upto 10 mL with DDW. For maximum colour development, the tube for left for 5 minutes and then was transferred to colorimetric tube. Optimum density was taken with spectrophotometer at 525 nm.

Standard curve for nitrogen

50 mg was added in one liter double distilled water. This solution was distributed in 10 test tubes (0.1 to 1.0 mL). In each test tube 5 mL double distilled water alongwith 0.5 mL Nessler's reagent was added. OD was taken in spectrophotometer at 525 nm after 5-6 minutes. For control, a blank solution was taken. With the help of different solution of ammonium sulphate standard curve was prepared.

Estimation of leaf phosphorus

Total phosphorous was estimated from leaf sample according to Fiske and Subbarow (1925). In this method, 5 mL aliquot was taken in a 10 mL graduated test tube and 1 mL of molybdic acid (2.5 % ammonium molybdate in 10 N H_2SO_4), was added carefully, followed by the addition of 0.4 mL of 1-amino-2-naphthol 1-4 sulphonic acid. The colour of the solution was turned blue. 10 mL volume was adjusted with DDW. Before transferring the solution in colorimetric tube the solution was shaken for 5 minutes and then at 620 nm on spectrophotometer, the optical density was taken. For control, a blank solution was taken.

Standard curve for phosphorus

In 1 liter of DDW, 351 gm of potassium orthophosphate and 10 ml of 10N sulphuric acid was added. From this solution, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 ml and 1.0 ml distribution in 10 different test tubes was done. Later, every test tube was filled with 5 ml DDW. 1 ml molybdic acid 0.4 ml 1-amino-2-naphthol 1-4 sulphonic acid was poured in each test tube. Optical density was taken at 620 nm on spectrophotometer after 5 minutes. For control, a blank solution was taken. Potassium dihydrogen orthophosphate solution was used for preparing standard curve.

Estimation of leaf potassium

Potassium was determined with the help of flame photometer. The leaf material was digested in hydrogen peroxide (10 ml) and for control, a blank solution was taken.

Standard curve for potassium

10 ppm potassium solution was prepared by dissolving 1.91 gm potassium chloride in 100 DDW and from this 1 ml solution was diluted in 1 liter water. This solution was separated in 1-10 ml solution in 10 different vials and then every vials were diluted with 10 ml DDW. Every diluted vials were run separately. For control, a blank solution was taken for each set. Through dilution of potassium chloride, standard curve was prepared.

Results and discussion

The use of natural products are ecofriendly economically viable for enhancing crop health and very effective for pathogens control for a long period. However, their field effectiveness is very low and presently, there is a very little information available on this aspect. To this end, further screening of more botanicals whose impacts can withstand different environmental complexities is of utmost significance, essential and made in this study. The most significant results particularly with respect to cane yield in brief associated with leaf nutrient (NPK) content (Tables 3 to 12) have been discussed on key points below.

It was observed that *Psidium guajava* (T₂) extract was found significantly most effective (16.44 % more) for cane yield which was statistically equal to *Phyllanthus niruri* (T₉) leaf extract followed by *Cassia tora* (T₅) as compared to control sprayed with water only (Table 12), which might be due to high potency of antipathogenic properties of these botanicals (Tables 1 and 2) noted in vitro conditions. However, it was surprising to note that disturbed leaf nutrient (NPK) content at all (240, 300 DAP and at harvest) stages (Tables 3 to 11) which is one of the most common effects of plant disease (Huber 1978; Gupta et al. 2018). Leaf nutrient components variations may be regarded as mini-imitation of the concentration

levels present in the stem tissues. The higher nitrogen content in the control (T_1) plants sprayed with water only shows accumulation of nitrogen in the red rot susceptible varieties. However the magnitude of accumulation varied according to the degree of resistance of cane varieties Co0238 (V_2) accumulated higher nitrogen content followed by CoPk 05191 (V_1) looking more prone to red rot disease, however, application of botanicals extracts against the test pathogen shows changed scenario with proper leaf nitrogen regulation at all stages of growth (Tables 3 to 5) which was probably associated with impact of their secondary metabolites respectively in creating tolerance and strengthening aggressively resistance to red rot disease (Tables 3 to 5). Similar results have also been noted by Huber (1978); Rao & Sharma (1978); Kumar et al. (1999, 2000); and Gupta et al. (2018). The lower phosphorus and potassium leaf content in these untreated plants (T_1) was due to accumulation of nitrogen content (Humbert 1963). These findings are in conformity with the observation made by Rao et al. (1984) and Kumar et al. (2000). As far as the interaction effect was concerned, varieties Co0118 (V_3), Co0238 (V_2) and CoJ64 (V_4) in combination with *Phyllanthus niruri* (T_9), *Psidium guajava* (T_2) and *Cassia tora* (T_5) gave highest and statistically equal values for cane yield (Table 12) as well as leaf nutrient (NPK) contents at almost all stages of growth (Tables 3 to 11).

Hence, it may be concluded that this ecofriendly, cost effective, sustainable, antipathogenic approach in enhancing sugarcane crop yield of red rot susceptible varieties could be employed extensively and can be recommended to farmers by further test trials with these as well as screening some more botanicals against the test pathogen that may act as leads in the development of new horizons of phytopharmaceutical research.

Table 3: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf nitrogen content (%) of sugarcane varieties at 240 days

(Mean of three replicates)

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	Mean
V_1	0.98	0.92	0.80	0.83	0.90	0.88	0.92	0.84	0.92	0.89
V_2	1.10	1.01	0.82	0.80	0.98	1.00	0.98	0.86	0.96	0.95
V_3	1.01	0.98	0.94	0.90	0.96	0.94	0.92	0.82	0.96	0.90
V_4	0.92	0.88	0.88	0.79	0.86	0.84	0.86	0.78	0.88	0.85
Mean	1.00	0.94	0.82	0.80	0.93	0.92	0.92	0.83	0.93	

CD at 5 %

$$T = 0.012^*$$

$$V = 0.008^*$$

$$TxV = 0.024^*$$

*Significant

Table 4: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf nitrogen content (%) of sugarcane varieties at 300 days

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	1.70	0.98	1.50	1.10	1.05	1.56	1.60	1.55	1.00	1.34
V ₂	1.99	1.10	1.80	1.19	1.04	1.76	1.80	1.78	1.20	1.52
V ₃	1.42	1.12	1.30	1.20	1.10	1.36	1.28	1.30	1.05	1.24
V ₄	1.40	1.00	1.38	1.12	1.10	1.24	1.26	1.30	1.00	1.20
Mean	1.63	1.05	1.50	1.15	1.07	1.48	1.49	1.48	1.06	

CD at 5 %

$$T = 0.012^*$$

$$V = 0.008^*$$

$$TxV = 0.024^*$$

*Significant

Table 5: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf nitrogen content (%) of sugarcane varieties at harvest

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	0.98	0.96	0.96	0.98	0.96	0.98	1.00	0.98	0.98	0.98
V ₂	1.02	0.98	1.00	1.01	1.00	1.01	0.98	0.98	1.00	1.00
V ₃	1.00	0.96	0.98	0.96	0.99	0.98	0.999	0.96	0.98	0.98
V ₄	0.98	0.90	0.92	0.94	0.88	0.92	0.90	0.92	0.90	0.91
Mean	0.99	0.95	0.97	0.97	0.96	0.97	0.97	0.96	0.97	

CD at 5 %

T = 0.011*

V = 0.007*

TxV = 0.023*

*Significant

Table 6: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf phosphorus content (%) of sugarcane varieties at 240 days

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	0.72	0.90	0.82	0.80	0.90	0.88	0.84	0.82	0.88	0.84
V ₂	0.80	0.88	0.84	0.86	0.90	0.88	0.84	0.84	0.90	0.85
V ₃	0.78	0.94	0.92	0.90	0.94	0.96	0.86	0.86	0.96	0.90
V ₄	0.81	0.98	0.94	0.92	0.98	0.96	0.90	0.90	0.96	0.93
Mean	0.77	0.93	0.88	0.87	0.93	0.92	0.86	0.86	0.93	

CD at 5 %

T = 0.012*

V = 0.008*

TxV = 0.024*

*Significant

Table 7: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf phosphorus content (%) of sugarcane varieties at 300 days

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	0.61	0.73	0.75	0.71	0.69	0.71	0.73	0.71	0.73	0.71
V ₂	0.59	0.71	0.69	0.68	0.71	0.68	0.71	0.69	0.67	0.68
V ₃	0.51	0.63	0.61	0.63	0.63	0.63	0.61	0.63	0.63	0.61
V ₄	0.58	0.71	0.69	0.71	0.73	0.69	0.70	0.69	0.73	0.70
Mean	0.57	0.70	0.69	0.68	0.69	0.68	0.69	0.68	0.69	

CD at 5 %

$$T = 0.013^*$$

$$V = 0.007^*$$

$$T \times V = 0.023^*$$

*Significant

Table 8: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf phosphorus content (%) of sugarcane varieties at harvest

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	0.34	0.52	0.42	0.40	0.50	0.50	0.52	0.39	0.48	0.45
V ₂	0.38	0.54	0.40	0.42	0.54	0.52	0.52	0.38	0.54	0.47
V ₃	0.40	0.56	0.46	0.48	0.55	0.55	0.56	0.45	0.56	0.51
V ₄	0.38	0.54	0.44	0.46	0.56	0.54	0.54	0.44	0.56	0.50
Mean	0.37	0.54	0.43	0.44	0.54	0.53	0.54	0.42	0.54	

CD at 5 %

$$T = 0.014^*$$

$$V = 0.009^*$$

$$T \times V = 0.029^*$$

*Significant

Table 9: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf potassium content (%) of sugarcane varieties at harvest

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	1.00	1.60	1.01	1.19	1.60	1.10	1.62	1.10	1.64	1.33
V ₂	1.11	1.80	1.24	1.20	1.80	1.15	1.80	1.28	1.80	1.48
V ₃	1.06	1.38	1.20	1.24	1.40	1.18	1.32	1.10	1.34	1.25
V ₄	1.00	1.39	1.10	1.22	1.39	1.20	1.40	1.16	1.38	1.25
Mean	1.00	1.54	1.14	1.21	1.55	1.16	1.54	1.16	1.54	

CD at 5 %

T = 0.011*

V = 0.007*

TxV = 0.023*

*Significant

Table 10: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf potassium content (%) of sugarcane varieties at 240 days

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	1.10	1.89	1.40	1.32	1.84	1.30	1.28	1.88	1.86	1.55
V ₂	0.98	1.94	1.34	1.36	1.90	1.28	1.40	1.90	1.92	1.56
V ₃	1.12	1.62	1.36	1.44	1.56	1.40	1.38	1.60	1.54	1.45
V ₄	0.99	1.56	1.34	1.38	1.54	1.34	1.32	1.48	1.56	1.38
Mean	1.02	1.74	1.36	1.38	1.71	1.33	1.35	1.72	1.72	

CD at 5 %

T = 0.048*

V = 0.032*

TxV = 0.096*

*Significant

Table 11: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on leaf potassium content (%) of sugarcane varieties at 300 days

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	2.00	3.80	3.00	3.38	3.32	2.99	3.01	3.00	3.34	3.09
V ₂	1.10	2.99	2.01	2.48	2.90	2.02	2.10	2.10	3.65	2.37
V ₃	1.90	2.99	2.89	2.50	2.60	2.79	2.80	2.86	3.44	2.75
V ₄	1.78	2.84	2.69	2.40	2.88	2.68	2.72	2.70	3.40	2.67
Mean	1.69	3.15	2.64	2.40	2.92	2.62	2.65	2.66	3.45	

CD at 5 %

T = 0.37*

V= 0.14*

TxV= 0.74*

*Significant

Table 12: Screening of different antipathogenic ethanolic leaf extracts (15 % conc) on cane yield (q/ha) in sugarcane varieties at harvest

(Mean of three replicates)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	Mean
V ₁	80.80	91.00	90.00	89.00	92.80	90.20	91.80	89.50	91.70	89.53
V ₂	80.30	97.20	91.00	91.20	92.80	95.20	92.70	92.70	97.00	97.73
V ₃	83.20	97.80	90.50	90.20	98.20	97.00	95.20	93.70	99.20	95.22
V ₄	83.60	95.80	91.70	90.30	91.00	91.00	90.30	93.50	93.70	89.39
Mean	81.97	95.45	90.80	90.17	93.70	93.37	92.50	92.22	95.40	

CD at 5 %

T = 1.45*

V= 0.98*

TxV= 3.98*

*Significant

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Chapter: 8***Rigidoporus vinctus*, an endophytic fungus with antioxidant and antimicrobial properties, from the shoot of *Bambusa tulda*.****Karthik. M.B^{1*} and Jayashankar. M²****¹Research scholar Department of Studies and Research in Microbiology, Mangalore University, Post Graduate Centre, Jnana Kaveri campus, Kodagu-571201, Karnataka, India****²Professor Department of Studies and Research in Microbiology, Mangalore University, Post Graduate Centre, Jnana Kaveri campus, Kodagu-571201, Karnataka, India****E-mail: karthikmbelliappa1@gmail.com**

Abstract: The recent exploration and investigation of various plants for bioactive potential have led to a growing interest to explore their endophytes. Microorganisms which colonize in the tissue of host plants are known as Endophytes. Endophytic fungi have been investigated as biofactories of novel bioactive substances. Bamboo shoot are highly nutrient and contains medicinal components, their endophytes are less explored effort was made to isolate endophytic fungi from the shoot. *Rigidoporus vinctus* is an endophytic fungus isolated by edible shoots of *Bambusa tulda*, from Billigirirangana hills, Chamrajanagara, Karnataka. Isolated fungi are identified by the 18S rRNA partial gene sequencing method. ITS region obtained are compared with closely related species from the NCBI database. The isolated fungal culture was subjected to mass culture by submerged fermentation method for the production of extracellular Bioactive compounds. The Solvent extraction method uses water, ethyl acetate, methanol, and chloroform. The acquired extract was screened for phytochemicals- Alkaloids, Flavonoids, Saponins, Tannins, Phenols, and Glycosides. The positive extract was analysed for antioxidant activity by DPPH and FRAP assay, Antagonistic activity by agar well diffusion assay against clinical pathogens- *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi* and *Klebsiella pneumoniae* obtained from KIMS Madikeri. The obtained sequences of *Rigidoporus vinctus* are submitted to NCBI with accession number ON287260. A phylogenetic tree is constructed using MEGHA 11 software. *Results* showed that *Rigidoporus vinctus* is a potential fungus for the production of numerous bioactive compounds. Ethyl acetate is a highly efficient solvent for extracting active compounds, then water, methanol, and chloroform. Extracts confirm the presence of phenols, alkaloids and flavonoids. Ethyl acetate extract has significant antibacterial and antioxidant capacity. This endorsed *Rigidoporus vinctus* as a promising drug development candidate.

Keywords: 18S rRNA sequencing, Bamboo shoot, antioxidant, bioactive compound.

Introduction

Endophytic fungi are fungi that form a mutually advantageous symbiotic connection with their host plant and survive in plant tissues for the duration of their complete or a portion of their life cycle without creating any negative effects or diseases.¹ Chemists, ecologists, and microbiologists have taken a keen interest in endophytic fungal habitats as a potential source of biological resources. Abiotic and biotic stress tolerance, eco-adaptation, promoting expansion and growth, and maintaining the health of their host are just a few of the amazing functions that endophytic fungi play in the system.² Other people emphasise the use of endophytic fungi as biocontrol agents. Plant responses to endophytic fungus invasion are also advantageous to the plant's immune system. As a result of the manipulation of direct antimicrobial metabolites such as alkaloids to indirect phytohormones, jasmonic acid, or salicylic acid, it has previously been shown that this endophytic interaction imposes additional defensive mechanisms on the control of the plant immune system.³ The biological activity of endophytic fungi's compounds are frequently observed to correspond to that of its host plant's compounds because of the endophytic fungi's extraordinary capacity to create chemical compounds.⁴ Over 136 bamboo species in 75 genera have been found in India, and of them, 25 are considered to be edible and are eaten by tribal groups. Bamboo has multidimensional uses like building material, food and they are known as green gold. The young, fresh bamboo shoots can be consumed as a tasty and healthy cuisine. It is abundant in vitamins, minerals, decreasing sugar, fibre, proteins, carbohydrates, and fibre⁵. Shoots have therapeutic potential against cancer⁶.

Materials and Method

Sample collection and processing

Edible shoots of *Bambusa tulda*, are collected from Billigirirangana hills, Chamrajanagara, Karnataka. During July month the shoots are brought to laboratory within 24 hrs in a clean sterile polythene bag. The shoots upper culm sheath was removed. To eliminate surface soil particles, germs, and adherent detritus, the edible parts are first washed in tap water for five minutes. They are then cleansed once more with distilled water.

Isolation of endophytic fungi

Shoot segments were submerged in 70% ethanol for three minutes, twice in 2% sodium hypochlorite aqueous solution for five minutes, and then once more for one minute. The

shoot samples should then be washed twice in sterile distilled water for 5 minutes to remove the surface sterilising agents, and then rinsed 6-8 times with sterile water in a laminar air flow hood further dried in the sterilized paper in a laminar air flow hood. The segments are later placed in an SDA Media (Sabouraud Dextrose Agar media) at room temperature for 3-7 days and the formation of conidia/mycelia were observed pure culture is obtained

Molecular identification 18S rRNA partial gene sequencing

In summary, the isolated fungi's genomic DNA was recovered using the CTAB method from their pure culture. ITS rDNA was amplified with forward primer ITS-1: 5'-TCCGTAGGTGAACATGCGG-3' Reverse primer ITS-4: 5'-TCCTCCGCTTATTGATATGC-3' and the Sequences were compared with fungal ITS sequences in GenBank using BLAST searches.

Submerged fermentation

Submerged fermentation was used to culture the endophytic fungi for the production of secondary metabolites from the chosen endophytic fungus isolates. Freshly subcultured fungi were then put into Erlenmeyer flasks filled with 500ml of Sabouraud Dextrose Broth (SDB) that had been autoclaved. The flasks were then incubated for 21 days at room temperature with constant shaking.

Solvent extraction

The fungal metabolites were extracted using the solvent extraction method. After harvesting the fermented fungal biomass on the 21st day, the fermentation broth was filtered through a sterile muslin cloth. The obtained filtered broth was divided into 4 parts around 100 ml in each flask, an equal amount of ethyl acetate, water, chloroform, and methanol solvent was added separately for the broth and they are transferred to a separating funnel and agitated 15min. The organic phase was collected and the solvent was removed by evaporation under reduced pressure at 45°C using a rotary vacuum evaporator. The dried crude extracts were weighed, labelled in separate vials, and then stored at 4°C for various biological attributes.

Phytochemicals analysis

In order to identify the chemical nature of the active components present in the endophytic fungal crude extracts, phytochemical analytical tests were performed to detect the presence of

chemical constituents such as Alkaloids, Flavonoids, Saponins, Tannins, Phenols, and Glycosides according to the method described by Banu and Cathrine ⁷.

Antagonistic activity

The dried crude secondary metabolites isolated from endophytic fungi were screened for their antibacterial activity against clinical pathogenic bacteria using Agar well diffusion method slightly modified described by Jayashankar⁸. Four clinical pathogens were used for antibacterial tests *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi* and *Klebsiella pneumoniae* which are obtained from KIMS Madikeri, Kodagu. Stock solutions (1mg/mL) were prepared by dissolving endophytic extract in Dimethyl sulfoxide (DMSO). A Loopful of test bacterial culture from the slant was inoculated in 3 ml nutrient broth in a test tube and incubated at 37°C for 24 hours. Nutrient agar medium was prepared, autoclaved and then cooled to room temperature. 100 µL (~10⁷ CFU/mL) of freshly prepared bacterial culture were swabbed and evenly distributed on the surface of nutrient agar plates. Wells were then made in the media with the help of a 6mm diameter sterile metallic borer. 100 µL of each of these endophytic fungal extract samples were added to the respective wells. Other wells were supplemented with DMSO (100 µL of pure DMSO) as negative control and reference antimicrobial drug streptomycin (1mg/ml) as a positive control. The plates were then incubated at 37°C overnight. Results were noted as inhibition zone diameter (IZD) in 'mm'.

Antioxidant activity by DPPH and FRAP assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2- picryl hydrazyl (DPPH) free radical was determined by the method described by Sethi *et.al*, ⁹. 1ml of each extract was added to 3ml of methanolic solution of DPPH (0.3mM) and were shaken vigorously. This was followed by incubation for 30min in complete darkness at room temperature and then the optical density was measured at 517 nm with ascorbic acid as a positive control. The results are recorded as mean values. All experiments were carried out in triplicate (n=3) and results are reported as mean values.

$$\%DPPH = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

FRAP (Ferric Reducing Power Assay)

Ferric reducing antioxidant power assay was measured according to the procedure described by Sethi *et.al*⁹ with some modifications. Different concentrations of the samples (1mL) were added to 2.5ml of phosphate buffer (0.2M) and 2.5ml of 1% potassium ferricyanide. Each mixture was then incubated at 50 °C for 20min and later 2.5ml of 10% trichloroacetic acid was added to each. The mixture was shaken vigorously, and the solution was mixed with 2.5 mL of distilled water and 0.5 ml 0.1% of Fe Cl₃. After 30 min of incubation, absorbance was read at 700nm. Ascorbic acid was taken as a standard reference, and each analysis was carried out in triplicates. Increased absorbance of the reaction mixture indicates stronger reducing power.

Results and Discussion

Endophytes that are connected with plants constitute a significant symbiotic relationship in nature and are well-established bio-reservoirs of natural compounds derived from plants. Endophytes colonise a plant's internal tissues without creating any visible alterations or disease symptoms. Because of their favourable impact on the formation of new metabolites with pharmacological importance, endophytes have attracted increasing attention in recent years¹⁰. Bamboo shoot are highly nutrient and contains medicinal components, The leaves have been utilised in traditional medicine for treating inflammation and blood disorders. For leprosy, asthma, and TB, the hardened material inside bamboo has been employed. The stalks are supposedly a digestive help and appetite stimulant. For ringworm, the root has been utilised. Earaches and deafness have both been treated with flower juice. Its effects on the uterus as well as its anti-inflammatory and anti-tumour properties have been supported by certain recent studies¹¹. By these properties of bamboo shows high interest to expose their endophytes which are less explored effort was made to isolate endophytic fungi from the edible shoots of *Bambusa tulda*, from Billigirangana hills, Chamrajanagara, Karnataka.

The shoot segments obtained are surface sterilized to avoid contamination and they are placed on SDA Media (Sabouraud Dextrose Agar media) plates later they are incubated at room temperature for 3-7 days. Plates are observed for the growth of fungi from the sides of shoot segments formation of conidia/mycelia were observed pure culture is obtained by subculture technique. A filamentous white circular growth pattern is observed in Fig 1. below microscopic view shows conidia and hyphae.

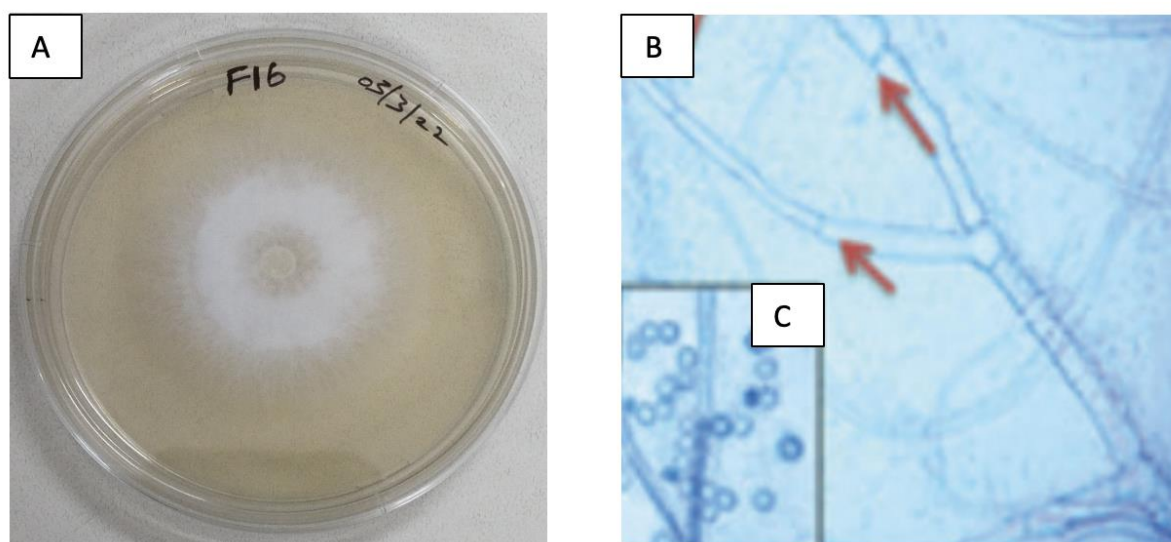


Figure 1. Photograph of A- Endophytic fungal growth on SDA media plate, B- Microscopic observation of Fungal hyphae, C- Enlarged microscopic view of Conidia.

Isolated fungi are identified by the 18S rRNA partial gene sequencing method. The acquired sequences are then altered and analysed using bio edit software. ITS region obtained are compared with closely related species from NCBI data base. The obtained sequences are as follows

18S rDNA partial sequence of fungi isolate F-16

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TTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGAATTTTATGGCGGAATTGTAGCTGG
CCCCAACCGGGCATGTGCACATTCTGTTTCATTCCATTCTCATACACCTCTGTGCACTTTAC
ATAGGTTTGGTATAGAAAAGGTCTTTATTGACTTTGGAAATACGAGTACTGACCTATGCT
TTTATAAACGCTTCAGTTTTAGAAATGTCATCCGCGTATAACGCAATAAATACAACCTTCA
GCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAAACGTATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCATCTTGCGCCCTTTG
GTATTCCGAAGGGCATGCCTGTTTGAGTGTGATGTTATTCTCAATACCCCAAATCTTTGC
GGATAAGGGTGTGTTGGACTTGGAGGTTTTTGCAGGTAATGATTGTGTTACCAGCTCCTC
TTAAATGCATTAGCAGAGATAATACTGCTACTCTCCAGTGTGATAATTGTCTACACTGTT
AGTAGTGCGGTATAACAAAATGTCTATGCTTCTAATCGTCTTCGGACAACCTTTGACAAT
CTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAA
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These sequences shows 98.3% similarity to *Rigidoporus vinctus* strain MFLUCC17-0007 with accession no MG545057.1, Query coverage 99.9%. Then the results are analysed by using MEGA 11 software to determine the level of kinship. Phylogenetic trees are created using the kimura 2 model with the bootstrap method and neighbour-joining tree are generated

with 1,500 replications. Later the obtained sequences are submitted to NCBI with accession number ON287260 as *Rigidoporus vinctus* strain MBKF16. A phylogenetic tree is constructed using MEGHA 11 software shown in Fig. 2

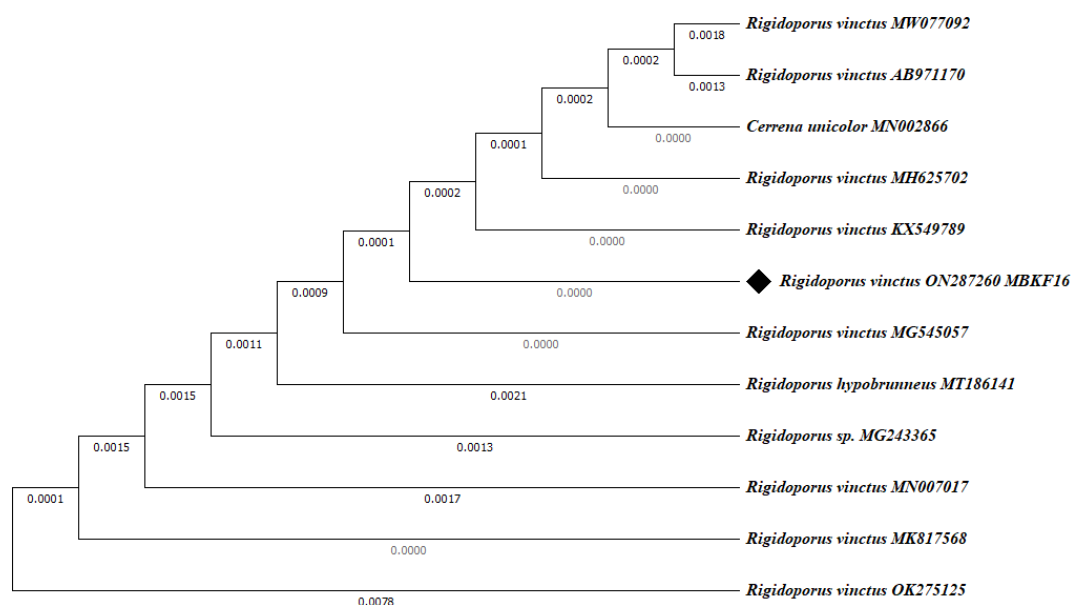


Figure 2: Phylogenetic tree of endophytic fungi strain MBKF16 isolate identified as *Rigidoporus vinctus*

Endophytes, which are present in plants and are able to produce metabolites and enzymes with comparable properties to those of the host plant extracts, are found in plants¹². The isolated fungal culture strain MBKF16 was subjected to mass culture by submerged fermentation method for the production of extracellular bioactive compounds. Freshly subcultured fungi were cultured in Erlenmeyer flasks filled with 500ml of Sabouraud Dextrose Broth (SDB) that had been autoclaved. The flasks were then incubated for 21 days at room temperature with constant shaking. Obtained broth with bioactive compounds are filtered and taken for solvent extraction method by using 4 solvents Water, Ethyl acetate, Methanol, and Chloroform. Acquired extract were screened for phytochemicals- Alkaloids, Flavonoids, Saponins, Tannins, Phenols, and Glycosides. Results are tabulated in (Table 1) clearly shows Ethyl acetate is an effective solvent for the extraction of phenols, alkaloids and flavonoids followed by water, methanol and chloroform.

Table 1- Phytochemical constituents of Endophytic fungal extracts

Sl no	Solvent used	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Glycosides
1	Methanol	+	++	+	+	++	+
2	Ethyl acetate	++	++	+	-	+++	-
3	Chloroform	+	+	-	+	+	+
4	Water	++	+	++	-	++	+

The result of the phytochemical analysis of the fungal extracts showed the presence of various secondary metabolites including phenolic compounds and flavonoids. These phytochemicals may be acting as antioxidants and anti-microbial components hence they are validated by following assays. Antibacterial activity of different solvent extracts was performed by agar well diffusion assay against clinical pathogens- *Escherichia coli*, *Shigella sonnei*, *Salmonella typhi* and *Klebsiella pneumoniae* obtained from KIMS Madikeri. Agar well diffusion assay is used to detect the capability of a substance to inhibit the growth of microorganisms by measuring the diameter of the inhibition zone around the compound on an agar plate in milli meters. Results are tabulated in (Table 2). Ethyl acetate extract shown highest antibacterial activity against *Escherichia coli* with high zone of inhibition with 16 mm and *Shigella sonnei* of 12 mm which shows the extract has compounds which have antibacterial components which can be used for drug development. literature states that *Rigidoporus vinctus* have anti-bacterial and anti-fungal properties¹³.

Table 2: Antibacterial activity of Endophytic fungal solvent extracts.

Sl no	The solvent used for extraction	<i>Escherichia coli</i>	<i>Shigella sonnei</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>
1	Methanol	8 ± 0.10	00	7 ± 0.16	00
2	Ethyl acetate	16 ± 0.15	12 ± 0.24	11 ± 0.18	10 ± 0.30
3	Chloroform	9 ± 0.22	00	00	7 ± 0.20
4	Water	12 ± 0.15	7 ± 0.10	11 ± 0.22	9 ± 0.28
5	Negative control DMSO	00	00	00	00
6	Positive control Streptomycin (1mg/ml)	18 ± 0.10	15 ± 0.10	15 ± 0.10	15 ± 0.10

Antioxidant activity- The DPPH and FRAP assays are two of the most common ones used to measure antioxidant capabilities. Oxidative stress is widespread in chronic degenerative

disease, dietary antioxidants may explain this protective effect. Every dietary plant contains a variety of antioxidants with varying characteristics. Many of these antioxidants work together to reduce oxidative stress. Plants and endophytes together contribute to the production of antioxidant compounds¹⁴. Based on the results, DPPH free radical scavenging activity of endophytic fungal extracts ethyl acetate extracts shows a high $69.15 \pm 1.88\%$ free radical scavenging activity.

Table 2: Antioxidant activity of Endophytic fungal solvent extracts

Sl no	The solvent used for extraction	DPPH Free radical scavenging activity (%)	FRAP Free radical scavenging activity (%)
1	Methanol	$9.10 \pm 1.22\%$	00
2	Ethyl acetate	$69.15 \pm 1.88\%$	$76.12 \pm 1.15\%$
3	Chloroform	$5 \pm 1.24\%$	00
4	Water	$45.18 \pm 1.25\%$	$38.12 \pm 1.13\%$
5	Negative control DMSO	00	00
6	Positive control Ascorbic acid	$98.12 \pm 1.10\%$	$95.20 \pm 1.09\%$

FRAP free radical scavenging activity of endophytic fungal ethyl acetate extract is $76.12 \pm 1.15\%$ which showed the highest free radical scavenging activity. By these results it clearly shows endophytic fungi *Rigidoporus vinctus* has a quality of producing bioactive compounds which help the shoots or Bamboo plant for defence mechanism against various pathogenic bacteria. It also contributes for the phytochemicals constituents of shoots directly or indirectly which increase the medicinal property.

Conclusion

The recent exploration and investigation of various plants for bioactive potential have led to a growing interest to explore their endophytes. Microorganisms which colonize in the tissue of host plants are known as Endophytes. Endophytic fungi have been investigated as biofactories of novel bioactive substances. Results showed that *Rigidoporus vinctus* is an endophytic fungus isolated by edible shoots of *Bambusa tulda*, from Billigirirangana hills, Chamrajanagara, Karnataka. *Rigidoporus vinctus* is a potential fungi for production of numerous bioactive compounds. Ethyl acetate is a highly efficient solvent for extraction of active compounds then water, methanol, and chloroform. Extracts confirm presence of

phenols, alkaloids and flavonoids. Ethyl acetate extract have significant antibacterial and antioxidant capacity which endorsed *Rigidoporus vinctus* to be a promising candidate for drug development.

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Chapter: 9**Investigation of bioactive molecules of *R. communis* by Gas Chromatography-Mass Spectrometry****Vijay. S¹* and Susikaran. S¹****¹Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam-641301*****E-mail: rsv8016@gmail.com**

Abstract: Castor plant (*Ricinus communis* L.) is the most important food plant for the eri silkworm which also produces castor oil and contains a variety of secondary metabolites. Castor plant leaves are also widely employed in many different industries owing to their wide range of biological components. This research used gas chromatography-mass spectrometry (GC-MS) to identify the bioactive compounds in a methanol extract of *R. communis* leaves. The chemicals identified by GC-MS in the methanol extract matched those in the National Institute of Standards and Technology database (NIST). GC-MS analysis of chloroform extract of *R. communis* leaves reveal the presence of bioactive compounds as N- Propargyloxycarbonyl-, tridecyl ester, Ethanol, 2-(9-octadecenyloxy)-, (Z)-, (S)-(+)-2-Amino-3-methyl-1-butanol and Methyl d- lyxofuranoside, Formic acid, 1,2-Benzenedicarboxylic acid, 2-propenyl ester, 2,2-di-n-Propylacetyl chloride, Stigmasterol, Octadecanoic acid and Phytol. Many biological processes are primarily carried out through these bioactive substances.

Keywords: Bioactive compounds, Biological activities, GC-MS, *R. communis*,

Introduction

Castor plant (*Ricinus communis* L.) is a flowering plant that belongs to the Euphorbiaceae family, which comprises a large number of tropical plants. It belongs to a monotypic genus *Ricinus* and cultivated all over the World with India, China, Brazil, Ethiopia, Paraguay, Vietnam and Thailand are its major grower by accounting 97 per cent of the World castor production (Deng *et al.*, 2023). The castor plant has a wide range of growth patterns, leaf and stem colours, seed size and colour, and oil content. Depending on the quantity of anthocyanin pigmentation present, the colour of the leaves fluctuates from light green to dark red (Huibo *et al.*, 2023).

Castor may be cultivated in both irrigated and rain-fed environments, in a wide range of climatic conditions, and practically on well-drained and low alkalinity soil (Cheema *et al.*, 2013). It is both a commercially significant plant for the production of industrial oil and primary feeding plant for the Eri silkworm, *Samia ricini* (Sarmah *et al.*, 2011). The selection of suitable host cultivar is an essential factor for improved Eri silkworm growth and development, as well as increased cocoon and egg production yield. The silk ratio varies dependent on the host and Eri silkworm breed utilized for rearing (Baruah, 2012). Narayanamma and Lakshmi (2018) stated that castor cultivation for Eri silkworm rearing and seed may work out the businesses to be more economical. Pharmacological studies are also being done on this plant's chemoprotective, antioxidant, antibacterial, and anti-inflammatory properties. The specific biological activities of the plant's chemical ingredients have remained elusive, and only a small number of substances found from *R. communis* have been employed as medicines (Yeboah *et al.*, 2020). Nonetheless, important components of the plant performed a wide range of biological tasks. In order to better understand the bioactive chemicals of *R. communis*'s leaves and their chemical composition and biological activity, this study used gas chromatography-mass spectrometry (GC-MS) analysis to classify each individual constituent according to its content.

Materials and Methods

Collection of leaf sample

Castor plant (*Ricinus communis* L.) leaves were taken from Tapioca and Castor Research Station, Yethapur. Geo co-ordinates at 11°35' N latitude, 78 °29' E longitude, 282 meters above Mean sea level (MSL) is situated in the agro climatic region of North Western Zone of Tamil Nadu whereas laboratory studies were carried out in the Department of Sericulture, Forest College and Research Institute, Mettupalayam, 11°19'N, 77°56'E and 320 m above MSL. The height of the plant was about 170- 200 cm grown under the warm condition in sandy loam soil. Leaf samples were collected from top, middle and bottom portion of the castor variety YTP 1 and kept as composite sample in a polythene bags.

Preparation of methanolic leaves extract for GC-MS analysis

Exactly 10 g of the crushed leaf sample of Castor was extracted with 250 mL of methanol in a soxhlet apparatus. The unit's sample tube was filled with ground samples, sealed with another filter disc, and compressed before being fitted with a filter disc at the bottom. This was mounted to a Soxhlet unit-equipped electric heating mantle, filled with 250 ml of

chloroform, and heated to 60 °C for six hours. In order to evaporate the solvent, the remaining extract was collected in a flask and transferred to a rotating flask vacuum evaporator (Luís *et al.*, 2016). Later it was dried at room temperature and dried extracts were stored at 4°C in air tight glass vials under refrigerated condition.

Gas Chromatograph and mass spectrophotometer (GC-MS) analysis

The chemical composition of methanolic leaf extract was analyzed using Gas Chromatography and Mass Spectrophotometer (QP 2020) with SH-Rxi-5 Sil MS Cross Band (similar to 5 % diphenyl 95 % dimethyl polysiloxane) capillary mid-polar column having 30m length 0.25 mm and film thickness of 0.25 µm. Sample size of 1.0µL methanolic extract was injected and Helium was used as carrier gas at 1.2 mL/minute. The column oven temperature was programmed from 80°C to 285°C (80°C for 5 min, 4°C rate 260°C, and 2°C rate 285°C hold for 10 minutes). The MS was set to scan from 45-650Da.

Results:

GC-MS analysis

The mass-spectrum GC-MS results were decoded using the National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns. Comparing the spectra of the unknown components to the spectrum of known components stored in the NIST library. The names, molecular weights, and structures of the test materials' constituent parts were used to characterize them. The GC-MS analyses with methanolic leaf extract samples of castor variety YTP 1 revealed 11 secondary metabolites. The major compound identified were N- Propargyloxycarbonyl-, tridecyl ester (19.72 %), Ethanol, 2-(9-octadecenyloxy)-, (Z)- (14.739 %), (S)-(+)-2-Amino-3-methyl-1-butanol (13.209 %) and Methyl d-lyxofuranoside (12.928 %) @ 11.362 minutes retention time. The minor compounds include Formic acid, 1,2-Benzenedicarboxylic acid (2.285 %) under the retention time of 22.191 minutes, Formic acid, 2-propenyl ester (0.667 %) @ 4.739 minutes retention time. 2,2-di-n-Propylacetyl chloride (0.612 %), Stigmasterol (0.685) @22.191 minutes retention time, Octadecanoic acid (2.036 %) and Phytol (0.637 %) @ 25.332 minutes retention time were found to be least in peak area (Table 1 and Fig 1).

Chemical structure and its biological properties

Eleven discovered compounds from the extract of *R. communis* leaves are shown in Table 2 and are reported in Dr. Duke's Phytochemical and Ethnobotanical Databases (1992-2016). These compounds have distinct biological capabilities and different chemical structures. According to the compounds' activities during GC-MS analysis, the specific medicinal applications are justified. Consequently, different biological functions of found bioactive compounds include analgesic, antipyretic, hepatoprotective, anti-asthmatic, and carbon metabolism. They also have antimicrobial, antiviral, and wound healing activity antioxidant, anti-inflammatory, and anticancer properties.

Discussion

The identified compound of methanolic leaf extract viz. D-Alanine, N-propargyloxycarbonyl-, tridecyl ester, Octadecanoic acid, Phytol, D-Alanine, N-propargyloxycarbonyl-, tridecyl ester and Ethanol, 2-(9-octadecenyloxy)- possess potent in anti-inflammatoroty, anti-hypertentive, antihyperglycemic, antimicrobial properties and antioxidant activities as revealed by other researchers. These results are in agreement with Carolina *et al.* (2019) reported the main compound in the methanolic extract of castor leaves were ricinine and Ethanol, 2-(9-octadecenyloxy)- which exhibited an antifungal activity. This finding is comparable with the reports of More *et al.* (2014) who opined that *Ricinus communis* leaves extract contains effective active phytochemicals responsible for eliminating pathogenic bacteria of *Staphylococcus* and *Escherichia coli*. Awa *et al.* (2012) also reported that Octadecenoic acid (Oleic acid; Elaidi acid) had antimicrobial activity against bacterial.

This research work confirm the finding of Hussein *et al.* (2015) that castor leaves ethanolic extract consisted of *n*-hexadecanoic acid, octadecanoic acid, 1- hexadecanoic acid, L-valine, ethyl ester, hexadecamethyl, tetradecamethyl, octadecamethyl, butanadioic acids, hydroxyl and diethyl ester. *R. communis* leaf extracts were shown to be highly concentrated in phenolic compounds, flavonoids, and alkaloids by performing phytochemical evaluation. Rukshana *et al.* (2017) reported that, leaf sample of *Pergularia daemia* identified compounds such as Hexadecanoic acid, Pentadecanoic acid, methyl ester, Ethyl 9,12,15-octadecatrienoate, 14-methyl-methyl ester and 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole were found to antiasthmatic and antimicrobial activities. Aziz *et al.*, (2016) reported that, GC-MS analysis of castor seed oil identified 9-Octadecenoic acid, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, Glycine,N-[(3a,5a)-24-oxo-3-

[(trimethylsilyl)oxy]cholan-24-yl]-,methyl ester compounds having the antifungal, antioxidant and antibacterial activity.

Santos *et al.*, (2018) were documented that, metabolites from stem, leaf and root of castor plant having antifungal and antibacterial activity. Antimicrobials made from plants have a great deal of therapeutic potential since they can do the same thing with fewer negative effects. Nowadays, there is a need for further research into plant-derived antibiotics. Phytochemical analysis of the extracts of *R. communis* leaves showed that they were rich in phenolic compounds, flavonoids, and alkaloids.

Conclusion:

Consequently, this kind of GCMS analysis is important for future research and is the first step towards determining the nature of *R. communis* bioactive constituents. In the current study, the presence of valuable bioactive substances such as phytol, Octadecanoic acid, Stigmasterol, Phenol, 2,4-bis(1,1-dimethylethyl), D-Alanine, N-Propargyloxycarbonyl-, tridecyl ester, and Methyl d-lyxofuranoside was revealed by GCMS analysis in the methanol extract of *R. communis* leaves. On the other side, it will almost certainly result in favorable outcomes for future research when certain phytochemical compounds are isolated and subjected to biological activity. Hence, it was concluded that the bioactivities of *R. communis* leaves contained pharmacologically active compounds which may enhance their use as a traditional medicine and promote silkworm growth.

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Table 1: Analysis of *R. communis* leaves bioactive compounds using GC-MS

S. No.	RT	Name of the compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1.	3.474	(S)-(+)-2-Amino-3-methyl-1-butanol	C ₅ H ₁₃ NO	103.16	13.209
2.	3.819	Ethanol, 2-(9-octadecenyoxy)-, (Z)-	C ₂₀ H ₄₀ O ₂	312.50	14.739
3.	4.739	Formic acid, 2-propenyl ester	C ₃ H ₆ O ₂	86.09	0.667
4.	6.645	Phenol, 2,4-bis(1,1-dimethylethyl)	C ₁₄ H ₂₂ O	206.32	0.619
5.	8.120	2,2-di-n-Propylacetyl chloride	C ₈ H ₁₅ ClO	162.66	0.612
6.	11.362	Methyl d-lyxofuranoside	C ₆ H ₁₂ O ₅	164.16	2.928
7.	22.191	Stigmasterol	C ₂₉ H ₄₈ O	412.70	0.684
8.	22.371	1,2-Benzenedicarboxylic acid,	C ₈ H ₆ O ₄	166.13	2.285
9.	22.461	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.48	2.036
10.	22.596	D-Alanine, N-Propargyloxycarbonyl-, tridecyl ester	C ₂₀ H ₃₅ NO ₄	353.50	19.722
11.	25.332	Phytol	C ₂₀ H ₄₀ O	296.50	0.637

Table 2: Bioactive compounds' chemical structure and biological activities

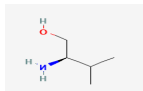
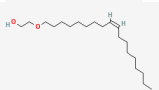
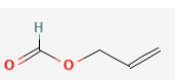
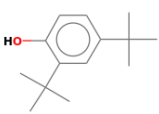
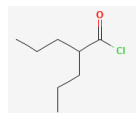
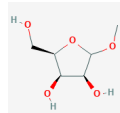
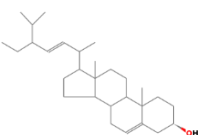
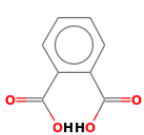
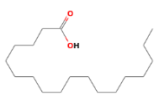
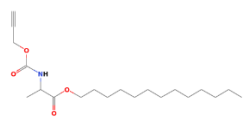
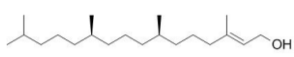
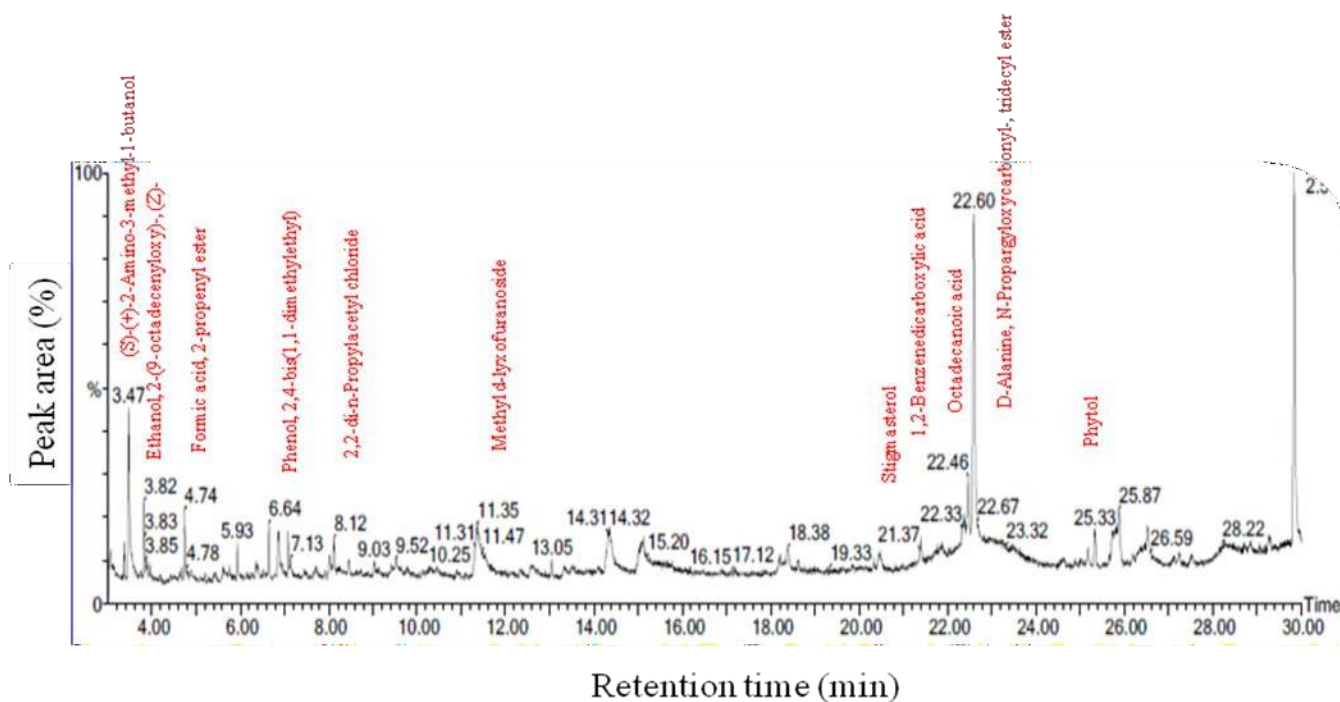
S. No.	Name of the compound	Chemical Structure	Functional Group	Biological activity
1.	(S)-(+)-2-Amino-3-methyl-1-butanol		Primary alcohols	Antibiotic
2.	Ethanol, 2-(9-octadecenyl-oxy)-, (Z)-		Alcohol	-
3.	Formic acid, 2-propenyl ester		Esters	Antifungal and antioxidant
4.	Phenol, 2,4-bis(1,1-dimethylethyl)		Phenols	Antioxidant, antifungal, anticancer. Antibacterial
5.	2,2-di-n-Propylacetyl chloride		Acyl chlorides	Antibacterial
6.	Methyl d-lyxofuranoside		Methyl furanoside	Antimycobacterial
7.	Stigmasterol		Sterol	Anti-inflammatory and Immunomodulatory
8.	1,2-Benzenedicarboxylic acid		Pentanol	Cytotoxic activity
9.	Octadecanoic acid		Saturated fatty acid	Antioxidants, Hypocholesterolemic, Nematicide, and Pesticide
10.	D-Alanine, N-Propargyloxycarbonyl-, tridecyl ester		Esters	Antifungal and Antibacterial
11.	Phytol		Alcohols	Antioxidant

Figure 1: GC-MS chromatogram of *R. communis* leaf extract showing the peaks of bio-active compounds



Chapter: 10**Phytochemical analysis and evaluation of antioxidant activity in methanolic extracts of *Macrotyloma uniflorum* (Lam)****Ritu Mahajan*, Muskaan Sharma, Shajaat Hussain and Nisha Kapoor****School of Biotechnology, University of Jammu, Jammu (J&K)*****E-mail: ritufeb@gmail.com**

Abstract: *Macrotyloma uniflorum* locally known as kulth, is a minor legume belonging to Fabaceae family. It is an underutilized crop with high nutritious and ethno-medicinal importance. Seed of *Macrotyloma* are used in the treatment of uterine stones, asthma, bronchitis, piles, diseases related to eyes, and liver. Methanol extracts of seeds of *M. uniflorum* were screened for the presence of phytochemicals. Antioxidant activity was evaluated by free radical scavenging activity and Ferric ion reducing power assay. Phytochemical screening of extracts revealed the presence of phenolics, tannins, saponins, terpenoids and flavonoids. Phenolic content observed was 34.1 ± 0.004 mgGAE/g extract while the flavonoid content was 81 ± 0.04 mgQE/g extract in the methanol extract. All the extracts inhibited DPPH radical in a concentration-dependent manner although methanol extract showed highest inhibition as compared to n-Hexane extract. DPPH assay of methanol seed extracts revealed good antioxidant properties with IC_{50} values of 7.80 ± 0.15 μ g/mL while ferric ion reducing antioxidant power was observed to be 11.2 ± 0.048 μ mol/g dry wt $\pm$ SD Fe^{2+} reduced. The antioxidant activity in the seed extract is attributed to the presence of the identified phytochemicals. The reported phytochemical studies and antioxidant potential supports its traditional uses and thus *M. uniflorum* could be used as a natural antioxidant, preservative and medicinal agent in food and pharmaceutical industry.

Keywords: *Macrotyloma uniflorum*, antioxidants, phytochemicals, flavonoids, phenolics

Introduction

Plants being rich source of phytochemicals, provides protection to the human beings against several diseases and oxidative stress (1, 2). Horsegram (*Macrotyloma uniflorum* (Lam)) belonging to family Fabaceae, locally called as Kulth is a minor grain legume. It is native to southern Asia and in India the plant grows in northwestern Himalayas, Karnataka, Tamil Nadu and Andhra Pradesh. The seeds are consumed as sprouts, or as whole meal in India. The plant is a perennial climber that grows at a height of about 55-60 cms. The leaves are

trifoliate with petioles up to 7 cm long. The flowers creamish yellowish in colour and the pods are about 8 cm long, containing reddish-brown or black seeds of about 5-6mm in length. Horsegram seeds are deficient in essential amino acids like methionine and tryptophan, but are rich source of iron and molybdenum.

The seeds have medicinal properties against asthma, bronchitis, urinary discharges. Soup of seeds is used in the removal of urinary stones (3). Also the studies were reported for activities against anti-hypercholesterolemic effect, anti-microbial activity, antihelmintic activity, anti-diabetic and anti-oxidant (4, 5). The present study highlights presence of various phytochemicals and antioxidant activity in the seeds of horsegram.

Material and Methods

Collection of Plant Material and Extract preparation

Seeds of horsegram were collected from Jammu region of Jammu and Kashmir. The seeds were homogenized into fine powder, and stored in vials. The powdered seeds (40 g) were extracted with methanol (400 mL), followed by continuous shaking. Sequential filtration was carried out thrice for the extracts using Whatman filter paper No.1. The filtrate was collected in sterilized petriplates and airdried to get gummy extract, which was redissolved in 1 mL of DMSO and 9 mL of methanol to get a working solution and stored at 4° C for further experiments.

Quantitative Estimation

Phytochemicals in the *M. uniflorum* seed extracts were quantified to record the concentration of primary and secondary metabolites present in them.

Total Carbohydrate Content

Total carbohydrate content was estimated using 3, 5- Dinitrosalicylic acid) (6). 100µL of extract was taken in test tube and 900 µL of distilled water was then added followed by addition of 3 mL of DNSA reagent (1g of DNSA, 0.2 g of phenol, 0.5 g of sodium sulphite and 1g of NaOH was dissolved in 100 mL DNSA solution). Tube was incubated at 90°C for 5 minutes and 5 mL of distilled water was also added to it. OD was taken at 540 nm and dextrose was used as standard.

Total Protein Content

Total protein content was estimated using Lowry's method (7). Alkaline copper sulphate solution was prepared by mixing solution A (2% sodium carbonate in 0.1N NaOH) and solution B (0.5% copper sulphate solution in 1% sodium potassium tartarate) in the ratio of 50:1. Folin–Ciocalteu (FC) reagent was diluted with equal volume of water just before use. 5mL of copper sulphate solution was added to the extract and incubated for 10 min at room temperature, followed by addition of 0.5 mL FC reagent. Absorbance was recorded at 660 nm. BSA was taken as standard.

Total Phenolic Content

Total phenolic content was estimated using Folin-Ciocalteu reagent (FC) (8). To 1 mL of 1N FC reagent 100 μ L extract was added and the mixture was kept at room temperature for 5 min. Reaction was stopped by adding 1 mL of 20% of sodium carbonate solution. The tubes were further incubated in dark for 2 h and the absorbance was measured at 760 nm. Gallic acid was used as standard.

Total Flavonoid Content

Total flavonoid content was estimated using calorimetric method (9). 150 μ L of 5% sodium nitrite was added to the tubes containing 100 μ L extract. The tubes were incubated at room temperature for 5 min, followed by addition of 300 μ L of 16% AlCl_3 to the tubes and the tubes were allowed to stand at room temperature for 6 min. 1 mL of 1 M NaOH (4%). was added to the mixture along with 550 μ L of distilled water and absorbance was measured at 510 nm. Rutin was used as the standard.

***In Vitro* Antioxidant Activities**

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

DPPH reagent was prepared by mixing 18 mg of DPPH in 100 mL of methanol. The mixture was incubated at room temperature for 30 min. 4 μ L of the extract was added to 100 μ L of DPPH and to it 2 mL of acetate buffer was mixed. The tubes were incubated in the dark for 30 min at room temperature and the absorbance was calculated at 517 nm. Ascorbic acid was taken as positive control, methanol as negative control. BHT (Butylated hydroxytoluene) was used as a reference and the IC_{50} value was determined as $\mu\text{g mL}^{-1}$.

FRAP (Ferric Reducing Antioxidant Power) Assay

Reducing potential of extracts was assessed by FRAP assay FRAP reagent was prepared by dissolving 300 mmol L⁻¹ acetate buffer (pH 3.6), 10 mmol L⁻¹ TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) in 40 mmol L⁻¹ HCl and 20 mmol L⁻¹ FeCl₃. To 3 mL of the FRAP reagent, 100 µL of extract was added and the mixture was incubated at 37°C for 5 min. Absorbance was measured at 593 nm using FeSO₄ as the standard solution. Results are expressed as µmol g⁻¹ dry weight Fe²⁺ reduced.

Statistical analysis

All Experiments were conducted in triplicate and results are presented as the mean ± standard Error (SE).

Results and discussion

Secondary metabolites present in plant organs have several pharmaceutical properties that have a positive effect on human health (10). Plants rich in phenolics, flavonoids, alkaloid tannins and terpenoids have anti-inflammatory and anti-cancer potential (11).

Quantitative phytochemical analysis

Total carbohydrate and protein content

Legumes are rich source of nutrients and phytochemicals which have been known for their contributions to health, food and economy (12). Legume starches contain high content of amylose with less bioavailability and low glycaemic index which is beneficial for health and especially for the prevention of diabetes (13). Carbohydrate content recorded in present study was 27.7 mg/g in the methanol extract while Sudha et al., (14) reported carbohydrate content ranged from 51.9-60.9% and 56.8-66.4% in whole and dehulled horsegram seeds respectively. However, Bravo et al., (15) reported 36±1.17g starch per 100gdry matter in raw horsegram seeds. Legumes are rich sources of protein, with protein content ranging 16%–43%. (16). The total protein content observed in the methanol extract of seeds of *M. uniflorum* was 10.4 mg/g while Yadav et al. (17) recorded protein and oil content in the seeds of the wild horsegram which was 38.37 ± 1.03% crude protein.

Total phenolics and flavonoid content

Phenols and flavonoids are major health promoting substances (10). The total content of phenols observed in the present study was 34.1 ± 0.004 mgGAE/g extract while the flavonoid content was 81.0 ± 0.04 mgQE/g extract in the methanol extract. Misra and Pathan, (18)

recorded high polyphenol and tannin content in horse gram as compared to the other legumes like chick pea, mung gram, pigeon pea. They also reported loss of polyphenols in pressure cooked horse gram which was due to the thermal decomposition of these compounds.

Antioxidant activity

The presence of bioactive metabolites in plants helps in avoiding the damage caused by reactive oxygen species (19). The antioxidant activity of horse gram extracts was determined by DPPH and FRAP assay. Ferric reducing potential of the seeds was observed to be $11.2 \pm 0.048 \mu\text{mol/g dry wt} \pm \text{SD}$ Fe^{2+} reduced while the radical scavenging activity in extracts from horsegram seeds was observed to be with IC_{50} values of $7.80 \pm 0.15 \mu\text{g/ml}$. Similarly, Siddhuraju and Manian (20) observed good antioxidant activity in different extracts of horsegram seeds. Interestingly, they reported that 70% acetone extracts of dry-heated samples showed significantly ($P < 0.05$) higher hydroxyl radical-scavenging activity.

Conclusion

The present study reveals the use of horsegram seeds as potential sources of natural antioxidants with significant antioxidant potential. The high antioxidant content in the methanolic extracts is due to the presence of phenolics and flavonoids. So, the seeds can be used as potent source of secondary metabolites in herbal and pharmaceutical industries.

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Chapter: 11

Studies Evaluating the Effects of Various Light Wavelengths on the Germination of *Phaseolus Vulgaris* Seeds and the Biosynthesis of Photosynthetic Pigments

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Abstract: The present work was undertaken to study the effect of different wavelengths of light on seed germination, hypocotyl growth, mobilization efficiency and vigour index in kidney beans (*Phaseolus vulgaris*). The extent of inhibition of germination was found to be dependent on wavelength of light along with the incubation period. No germination was seen after 96 hours in green light. The average hypocotyls length was maximum in red light with 14.6 cm. Mobilization Efficiency and Vigour Index was maximum in red light with 685.72 ± 7.327 and 5.13 respectively. Blue light showed positive impact on pigment synthesis as compared to other treatments.

Keywords: *Phaseolus vulgaris*, Mobilization Efficiency, Vigour Index, hypocotyl, germination

Introduction

Light is an absolute requirement for growth and development. The various environmental factors controlling plant development, light plays an important role, in photosynthesis, in seasonal and diurnal time sensing, and as a cue for altering developmental pattern. Light signals can regulate changes in structure, such as seed germination (Scopel et al. 1994), leaf expansion, stem elongation, flower initiation and pigment synthesis. These photomorphogenic responses confer an enormous survival advantage on organisms. Light is important factors affecting the germination of seeds. The light environment that effect germination includes the length, quality and photon irradiance of the light reaching the seed of the crop.

Phaseolus vulgaris locally known as Rajma in India. It is a multiuse crop, but it has not been given the much priority in the selection among the rest of the pulses like, Mash bean, Mung bean, Chickpea (Chana) and Lentil (Masoor). Kidney beans (*Phaseolus vulgaris*) is one of the most important legumes, being cultivated mainly in the hilly areas and plains as well for

its grain and food purposes. Among all the legumes, kidney beans have great tolerance to drought, is a warm weather crop and hence well adapted to tropical and temperate regions. Kidney beans is an annual warm season, self-pollinated herbaceous crop grown as Kharif Legume in India. It has different plant types like erect, semi erect, prostrate (trailing) and climbing. The growth may be determinate or indeterminate. Most of the dicot plants are sensitive to different colour light. In spite of immense importance of the crop, there are few reports on effect of light on seed germination, hence the present work was designed to study the effect of different colour lights in this important leguminous plant with special reference to seed germination, hypocotyl growth, mobilization efficiency and vigour index in *Phaseolus vulgaris*. The present investigation would be of pivotal importance for future studies on the effect of light on plants in general and Rajma in particular.

Materials and methods

Experimental material

Rajma (*Phaseolus vulgaris*) was used as experimental for germination. Knop's solution was used as the nutrient medium for proper germination of seeds, all stock and treatment preparations were made in distilled water and comparisons were made using only distilled water treated plant seeds or seedlings.

Preparation of reagents

Knop's solution (10X)- 0.8 mg of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.2 mg of KNO_3 , 0.2 mg of KH_2PO_4 , 0.2 mg of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and traces of FeSO_4 was dissolved in 50 mL of distilled water and this was used for preparation of nutrient solution for germination of seeds.

Nutrient solution

25 mL of Knop's solution was taken in measuring cylinder and 25 mL of distilled water was added and then transferred to the beaker. Then 200 mL distilled water was added in the beaker to make final volume of 250 mL of nutrient solution.

Germination of seeds

Exposure of seeds to light

Seeds of *Phaseolus vulgaris* were grown in a series of 4 Petri dishes each with a bed of cotton and normal filter paper was moistened with 30 mL of nutrient solution. The petri dishes were

exposed to light of different wavelength i.e., natural light, red light, yellow light, green light and blue light.

Growth of experimental material

The effect of each light treatment was studied on 100 seeds of Rajma. The seeds were imbibed by immersing them in 30 mL of nutrient solution in glass Petri dishes. The covers of the petridishes were closed and determination the germination rate was done after every 12 hours. A 1.0 mm radical emergence from seeds was considered as proof of seed germination. At least 84- or 96-hours' time was provided for proper growth of seedlings. The seedlings were grown up to 96 hours in the petridish and observation were recorded for germination and seedling growth. Different seedling growth parameters (root length, shoot length, root fresh weight, shoot fresh weight, dry weight of seedling and cotyledon, mobilization efficiency and vigour index) were recorded at specified stages.

Visual observations

The time taking for initiation of germination and % germination data were recorded for every light with each treatment after every 12 hours.

Evaluating the biomass production

Biomass estimated was based on the fresh weight and % dry matter content at 84 or 96 hours. Fresh weight of plants/seedlings was measured after washing them with tap water and drying them with blotting paper.

Different seedling growth parameters

Ten seedlings were collected randomly from 4 petridishes after 96 hours and washed with tap water. The root and shoot portion were recorded for length and different parameters i.e., fresh, and dry weights. The following parameters were recorded at 96 hours duration:

- (a) Root length
- (b) Shoot length
- (c) Hypocotyl Length
- (d) Root fresh weight
- (e) Shoot fresh weight
- (f) Dry weight of seedling and cotyledon

For evaluating the dry weight of cotyledon and seedling, the cotyledon and seedling portion were separated and were placed on aluminium foil and were placed in hot air oven at 72°C for 48 hours in order to achieve constant dry weight.

Mobilization Efficiency (ME)

ME in germinating seedling for each treatment was calculated by the following formula (Mohan et al. 1996),

$$\text{ME} = \text{Dry weight of seedling} / \text{dry weight of cotyledon} * 100$$

Vigour Index (VI)

VI of resulting seedling for each treatment was calculated by the following formula (Abdulkali and Anderson, 1973),

$$\text{Vigour Index} = \% \text{ germination} * \text{average hypocotyl length}$$

Estimation of photosynthetic pigments

In Rajma after 5 days 80%(v/v) of acetone homogenate of plants was put into refrigerator for 24 hours at 4°C for complex extraction of pigment then the homogenate was centrifuged at 3000 revolution per minute for 15 min. The supernatant was made up to known volume and O.D. of each sample was determined at 480, 510, 630, 645, 652, 663 and 665 nm using spectronic 20 Bausch and Lomb model spectrophotometer. The content of Chl a, Chl b, total Chl and carotenoid were estimated in mg/g fresh weight according to method of Arnon (1949) with slight modification (Bansal et al. 1978).

Calculating the pigment values,

$$\text{Total Chl} = \text{O.D.}_{652} * 1000 / 34.5$$

$$\text{Chl a} = 15.6 * (\text{O.D.}_{665}) - 2 * (\text{O.D.}_{645}) - 0.8 * (\text{O.D.}_{630})$$

$$\text{Chl b} = \text{Total Chl} - \text{Chl a}$$

$$\text{Carotenoids} = 7.6(\text{O.D.}_{480} - 1.4 * \text{O.D.}_{510})$$

$$\text{Actual pigment content in mg/g. FW} = \text{Pigment value} * v / 100 * 1/W$$

Where,

v= volume of acetone in ml

W= weight of the tissue in g.

Results and discussions

Germination behaviour

In control at 12 hours incubation period, no germination was seen but after 24 hours the changes started taking place in seeds. After every 12 hours incubation period % of germination increased and at 84 or 96 hours germination stopped. The germination % gradually increased from 24 hours to 96 hours and same pattern was followed by other light treatments as well (Table 1). In natural light germination was fast with 94% at 84 hours. After natural light the germination is better in red light because its wavelength was found more suitable for germination of seeds. In red light germination was maximum (97%) at 84 hours. Under blue light germination was maximum (75%) at 84 hours and under yellow light germination was maximum (61%) at 84 hours. Under blue light germination was more in comparison to yellow light. Under green light almost no germination was seen even after 96 hours. In comparison to all other lights, green had negligible germination (was not supporting germination of seeds).

Table1 The germination rate of Rajma (*Phaseolus vulgaris*) in the presence of different light treatments.

Treatments	% Germination							
	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	84 hrs	96 hrs
Natural light	13	45	79	82	89	93	94	94
Red light	7	22	62	73	83	90	97	97
Blue light	9	47	50	59	62	64	75	75
Yellow light	6	23	54	58	59	60	61	61
Green light	*	*	*	*	*	*	*	*

* No Measurable Growth

Biomass production

Effects of different lights on length of root, shoot, hypocotyls and biomass (FW) of root and shoot portions of Rajma (*Phaseolus vulgaris*) is summarized in Table 2. The average hypocotyls length in natural light was (10.4 cm), in red light was (14.6 cm), in blue light was (10.48 cm) and in yellow light was (9.23 cm).

Table 2 Growth response of root, shoot and hypocotyls of germinating seeds of Rajma (*Phaseolus vulgaris*) under different light treatments

S. No.	Treatments	Root Length (cm)	Root FW (g)	Shoot Length (cm)	Shoot FW (g)	Seedling Dry wt.	Cotyledon Dry wt.	Hypocotyl length
1	Natural Light	2.151±0.239	0.021±0.003	3.256±0.175	0.191±0.015	0.022±0.006	0.019±0.004	1.958±0.213
2	Red Light	3.159±0.148	0.024±0.005	5.277±0.181	0.262±0.017	0.0026±0.004	0.026±0.006	2.312±0.197
3	Blue Light	1.453±0.192	0.009±0.004	0.672±0.061	0.194±0.010	0.013±0.002	0.015±0.002	1.520±0.210
4	Yellow Light	2.194±0.300	0.019±0.003	4.561±0.490	0.238±0.021	0.020±0.007	0.024±0.007	2.166±0.261
5	Green Light	*	*	*	*	*	*	*

*Parameters were not measurable

Mobilization Efficiency

Variation was recorded in mobilization efficiency of seedlings of Rajma (*Phaseolus vulgaris*) obtained in different light treatments (Table 3). ME was maximum in red light (685.72 ± 7.327) and minimum in yellow light (192.46 ± 3.013).

Vigour Index

Vigour index varied considerably in different tested lights (Table 3). VI depended upon germination from the initial stage to the final stage. It depends on average hypocotyls length of seedlings which are randomly selected in case of each treatment. VI was maximum under red light (5.13).

Table 3 Changes in ME efficiency and VI of Seedlings of *Phaseolus vulgaris* under different light treatments.

Treatments	Mobilization Efficiency (ME)	Vigour Index (VI)
Natural Light	605 $\pm$ 3.154	
Red Light	685.72 $\pm$ 7.327	5.13
Blue Light	621 $\pm$ 2.916	
Yellow Light	192.46 $\pm$ 3.013	
Green light		

*ME and VI could not be determined because no differential/well marked hypocotyl present.

Table 4 Photosynthetic pigment contents (in mg/g FW) of Rajma seedling leaves grown under different light treatments.

Treatments	Chl a	Chl b	Carotenoids	a: Chl b ratio	Chl: carotenoids ratio
Natural Light	1.2	0.8	0.5		
Red Light	1.5	1.0	0.6		
Blue Light	1.2	0.8	0.5		
Yellow Light	0.5	0.3	0.2		

*Chl- Chlorophyll, No measurable pigment content in green light

When the seedlings are formed under different lights, they show different photosynthetic pigment

contents in terms of Chl a, Chl b and carotenoids (Table 4). Under natural light treatment the value of Chl a was 0.489 mg/g and Chl b was 0.365 mg/g, respectively and carotenoids were 0.423 mg/g. In red light the value of Chl a was more rather than any other light treatment and carotenoids were 0.396 mg/g. Under blue light treatment the value of Chl a was 0.382 mg/g, Chl b was 0.302 mg/g and carotenoids were 0.301 mg/g respectively. Thereafter, treatment of seeds under yellow light, the carotenoids value was 0.298 mg/g and the values of Chl a was 0.323 mg/g and Chl b was 0.310 mg/g. There were no measurable pigment contents under green light treatment. The ratio of Chl a and Chl b under different lights were as follows- natural, red, blue yellow light were 1.33 mg/g, 1.79 mg/g, 1.26 mg/g and 1.04 mg/g respectively. The red light showed more pigment contents than any other light.

In different light treatments, we have observed different photosynthetic pigment contents. In red light, pigment contents were higher in comparison to other treatments and blue light had minimum pigment contents (Table 4). Green light had no measurable pigment contents because of insufficient growth and development of seedlings. The ratio of Chl a and Chl b was variable in different light treatments. In red light treatment the ratio of Chl a and Chl b was maximum (1.79 mg/g) and ratio of total Chl and carotenoids was higher (2.27 mg/g) in blue light in comparison to other lights.

It was revealed from this study that different light treatments can have various effects on different seed germination parameter of *Phaseolus vulgaris*. Light is an important factor affecting germination and seedling growth. Rajma seeds which were exposed to red and white light started to germinate within 12 hours after sowing. Research works conducted by Ellis and Robert (1981) and Hangarter(1997) reported that many plant species responded to the environment with optimal growth and development according to the light they received. Colbachet al.(2002) reported that some seeds germinated under different lights. In this experiment, the earliest germination was observed in white and red-light treatments.

Germination of seeds of *Ruellia tuberosa* (Borthwick, 1957), *Asteracantha longifolia* (Sen and Chawan, 1970) and *Cucumis melo* (Bansal and Sen, 1978) was promoted when irradiated with red light. These reports supported the findings of Sen and Chawan (1970) and Sharma and Sen (1975) that the red region of spectrum (590 and 680 nm) was most effective for the germination of light requiring seeds. In *Phaseolus vulgaris* seeds germination started 12 hours after sowing and seeds under blue light started to germinate under 24 hours. Sen and Chawan (1970) and Sharma and Sen (1975) also reported that seedling growth of some *Merremia* species was the least in blue light. This was analogous to the findings by Wareing and Black (1958) and Gwynn and Scheibe (1972) with respect to lettuce seeds. Sharma and Sen (1975) and Steel and Torrie (2010) reported that highest percentage of some *Merremia* species was found in the red light. After 84 hours, all the seedlings were counted for germination percentage. Seedlings under red light gave the highest percentage. Saebo et al. (1999) reported that red light was important for the development of the photosynthetic apparatus of plant and blue light was important in the formation of chlorophyll. Seedlings under red light gave the highest average germination percentage. Seedling vigour index and germination index were highest under red light, followed by yellow light. The maximum root length was observed on seedlings under red and in natural light.

Van et al.(1976) reported that the chlorophyll content of *Hydrilla* and *Vallisneria* plants grown in water 1.5 m deep. The low chlorophyll contents probably reflect the low percent dry wt. in submersed aquatic sp., being about 6 to 8 % as compare to 20 to 30 % in terrestrial plants. Further, *Hydrilla* and *Vallisneria* have lower Chl a: Chl b ratio than are found in terrestrial plants. The relatively higher proportion of Chl b in these species suggested that this pigment may be play a role in aquatic plant production probably by enabling the plants to more effectively use longer wavelength of light for photosynthesis. Dry weight yields were higher under red and blue light while natural and green light produced the lowest yield. The total chlorophyll contents were not affected by these lights regimes. However, the chlorophyll a: b ratio was lower in plants grown under green light than in other treatments.

Wolf (1963) reported the seeds of higher plants contain relatively small quantities of carotenoids pigments. Upon germination and early seedling growth in the absence of light more carotenoids are synthesized. The biosynthesis of carotenoid pigments which can occur in total darkness is greatly stimulated in light. In different light treatments, photosynthetic pigment contents were variable. In red light treatment, photosynthetic pigment contents were higher than other treatments and were recorded lowest in yellow light treatment. The ratio of Chl a and b was more in red light and the ratio of total chlorophyll and carotenoids was more in blue light treatments. So, the study shows that different pigments contents are present in seedling grown under different light treatments. Dry weight yields were higher under red and blue light while natural and yellow light produced the lowest yields. Seedling grown under green light have no effect on any parameter (root length, shoot length, hypocotyl length, mobilization efficiency, vigour index, photosynthetic pigment contents) due to lower wavelength in comparison to natural, red, blue and yellow light.

Conclusion

The data on root and shoot length and biomass revealed that the root length, root biomass, shoot length and biomass were maximum under red light treatment. Content of photosynthetic pigments (Chl a, Chl b, total Chl and carotenoids) were also variable in different light treatments. Blue light shows more effect on chlorophyll synthesis in comparison to all other treatments.

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Chapter:12

Utility of Biosensor Technologies in Early Detection of Plant Disease and Insect Pest Infestation

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Abstract

Detection and quantification of plant pathogens and pest are primarily the first procedure before the application of any strategy for combating them. Within the last few years; analytical biosensors have provided a promising alternative for direct disease and pest detection. A biosensor is an analytical device that integrates a biological element on a solid-state surface which enables a reversible biospecific interaction with the analyte, and a signal transducer. The biological element is a layer composed of molecules qualified for biorecognition, such as enzymes, receptors, peptides, single-stranded DNA, even living cells are applicable. Biosensor technology in general seeks to improve analytical performance by reducing the consumption of reagents, decreasing the analysis time, increasing reliability and sensitivity through automation, and integrating multiple processes in a single device. Biosensors can be used for easy detection of pathogenic micro-organisms (fungi, bacteria, virus, etc.) and pest infecting plants. Several biosensors such as Enzymatic biosensor, Bacteriophage-based biosensor, DNA based biosensor etc have been developed for detection of various plant diseases. Plant VOCs play an important roles in responding to pest attacks and are promising targets for pest detection. E-noses have been successfully demonstrated for detecting insect infested plants and insect population dynamics. Infected plants attacked by pest can be easily discriminated from healthy plants by E-nose.

Introduction

Before application of any management strategy early diagnosis and detection of plant diseases are usually the first step to get rid of them. In the last couple of years, different biosensors techniques have been used for direct plant disease detection within short period of time in comparison to other techniques that are time consuming. Biosensor technology can

be regarded as an improved analytical technique compared to conventional techniques, for early detection of plant diseases by exploiting the selectivity and sensitivity of biological systems in smaller and low cost devices. A biosensor is an analytical device that integrates a biological element on a solid-state surface which enables a biospecific reversible interaction with the target analyte, and a signal transducer in order to quantify or measure a compound or conditions. Biosensors combine the specificity of biological system with the processing power of modern microelectronics to offer a new sharpened analytical tool with major application in various fields viz; medicine, environmental diagnostic, food industries and agriculture. Agriculture mainly focuses on crop production and the livestock rearing which play a very important role in day today life. However, this always seems to be susceptible to certain plant pests and diseases which causes huge economic loss in agricultural production. Therefore increasing profits depend on reducing the crop loss by such natural damages. In such scenario, the biosensor can be used as a reliable technology for early pest and disease detection in order to reduce major economic losses in crop production. Moreover increase in advancement of bioterrorism, need for biosecurity becomes very essential, especially when agricultural products are transported across the international borders. The spores of, Ug- 99 race of *Puccinia graminis tritici* causing wheat rust and brown spot causing pathogen of rice *Dreclera oryzae* which are used for bioterrorism, causing large scale human destruction and famine can easily be detected by using biosensors. More research on biosensor has been focused for early detection of crop diseases and pest, agricultural by-products, contamination of soil diseases, biowar weapons, in order to prevent direct loss and damages caused by them.

Characterstics of Biosensor

There are positive static and dynamic attributes that each biosensor possesses. The optimization of those properties is pondered at the performance of the biosensor

- 1. Selectivity:** Selectivity is perhaps the most essential feature of a biosensor. Selectivity is the ability of a bioreceptor to hit upon a particular analyte in a sample containing different admixtures and contaminants. The best example of selectivity is depicted by means of the interaction of an antigen with the antibody. Classically, antibodies act as bio receptors and are immobilised at the surface of the transducer. A solution (commonly a buffer containing salts) containing the antigen is then exposed to the transducer in which antibodies have interaction handiest with the antigens. To assemble a biosensor, selectivity is the primary attention while choosing bio receptors.

- 2. Stability:** Stability is the degree of susceptibility to ambient disturbances in and across the biosensing system. Those disturbances can purpose a drift in the output signals of a biosensor beneath measurement. This can reason an errors in the measured attention and may affect the precision and accuracy of the biosensor. Stability is the maximum vital feature in applications where a biosensor calls for long incubation steps or non-stop monitoring. The response of transducers and electronics can be temperature-sensitive, which may additionally affect the stability of a biosensor. Consequently, appropriate tuning of electronics is needed to ensure a solid response of the sensor. Some other issue that may influence the stability is the affinity of the bioreceptor, that is the degree to which the analyte binds to the bioreceptor. Bioreceptors with high affinities inspire both sturdy electrostatic bonding and covalent linkage of the analyte that fortifies the steadiness of a biosensor. Another element that affects the stableness of a size is the degradation of the bioreceptor over a period of time.
- 3. Sensitivity:.** The minimal amount of analyte that may be detected by means of a biosensor defines its limits of detection (LOD) or sensitivity. In a number of scientific and environmental monitoring programs, a biosensor is needed to detect to analyte concentration of as low as ng/ml to verify the presence of traces of analytes in a sample.
- 4. Linearity:** Linearity of the biosensor may be associated with the resolution of the biosensor and variety of analyte concentrations under test . The resolution of the biosensor is described as the smallest alternate in the concentration of an analyte this is required to bring a exchange within the response of the biosensor. Depending on the application, an excellent decision is required as most biosensor applications require not simplest analyte detection but additionally size of concentrations of analyte over a wide working range. Another term related to linearity is linear range, that is defined as the range of analyte concentrations for which the biosensor response modifications linearly with the concentration.

Basic Principle Involved in Biosensor

The basic principle is based on binding of specific target of interest that is complementary to the molecularly recognizing element (cells, enzyme, antibody, and microorganism) immobilizing on a suitable support medium. This causes changes in pH, heat flow, transfer of electron, change in mass which are quantified and detected by the transducer. The concentration of the target analyte or mixtures of analyte is directly proportional in magnitude to the electric signal produce to which the element of biosensor binds (Turner *et al.*, 1986; Powner, 1997).

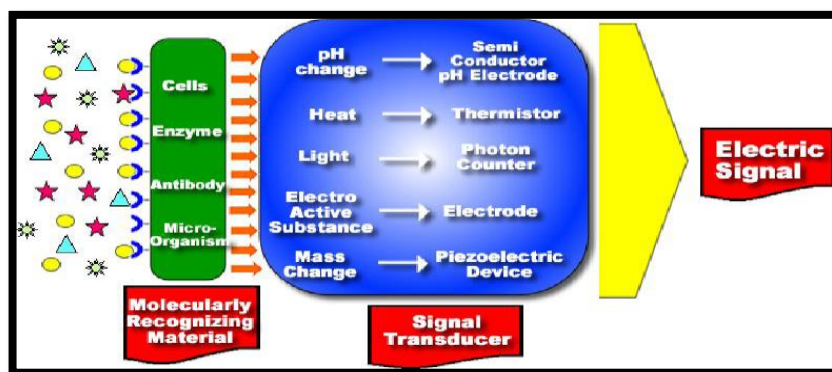


Fig1. Principle of Biosensor

Biosensors in plant pathology

Newly developed biosensor technique can help in early detection of plant diseases on-field that might help the farmers for easy diagnosis of diseases and specific application of pesticides against the diseases. Recently, antibodies based biosensor seems to be most preferred detection techniques since they can acts immonusensor for detection of plant pathogen and numerous pathogen specific antibodies are also available for quantification of a particular pathogen. The main application of biosensor in plant pathology are as follows-

1. Detection of various fungal, bacterial and viral plant pathogens
2. Detection and Enumeration of fungal spores
3. Detection of mycotoxins produced by fungus that are carcinogenic to human health

A biosensor has been designed using the Surface Plasmon Resonance technique for the detection of Soybean rust disease caused by the fungus *Phakopsora pachyrhizi*, an antibody was used as a biorecognition element in this technique. *Aspergillus flavus* and *A.parasiticus* produced a toxin- Aflatoxins that causes many chronical diseases which seems to be carcinogenic to human health. Many researcher have reported that aflatoxin can inhibit the activity of the enzyme acetylcholinesterase (AChE),therefore its

detection is measured using a choline oxidase based amperometric biosensor which is quantified with decrease in the activity of AchE. Biosensors can also be used for easy detection of harmful fungal spores.

Plant Disease Detection by Enzymatic Electrochemical Biosensors

Methyl salicylate (MeSa), a volatile organic compound released by plants can be regarded as the important electrochemical markers for early detection, quantification and identification of plant diseases and therefore can be regarded as potential strategy for combating biotic stress in plants.

Fang *et al.*, (2016) developed a tri-enzymatic biosensor that consists of salicylate hydroxylase (SH) esterase (ES), and tyrosinase (TYR) for detection and quantification of MeSa released by the infected plants. The release of MeSa is based on biocatalytic reaction produced on the electrode. As depicted in Fig2. the enzyme esterases firstly hydrolyzed MeSa in the electrolyte that results in generation of methanol and salicylic acid. This two compound was then quantified using an SH/TYR-immobilized screen-printed electrode (SPE). This reaction results in production of catechol which was then oxidized by enzyme tyrosine to produce an organic compound 1,2-benzoquinone. This electro active compound was then again reduced back to catechol on the surface electrode in order to generate reduction current which finally indicated the presence of pathogen in the plant tissue. Thus, the concentration of volatile compound MeSa produced is directly proportional to the reduction current collected on the cathode.

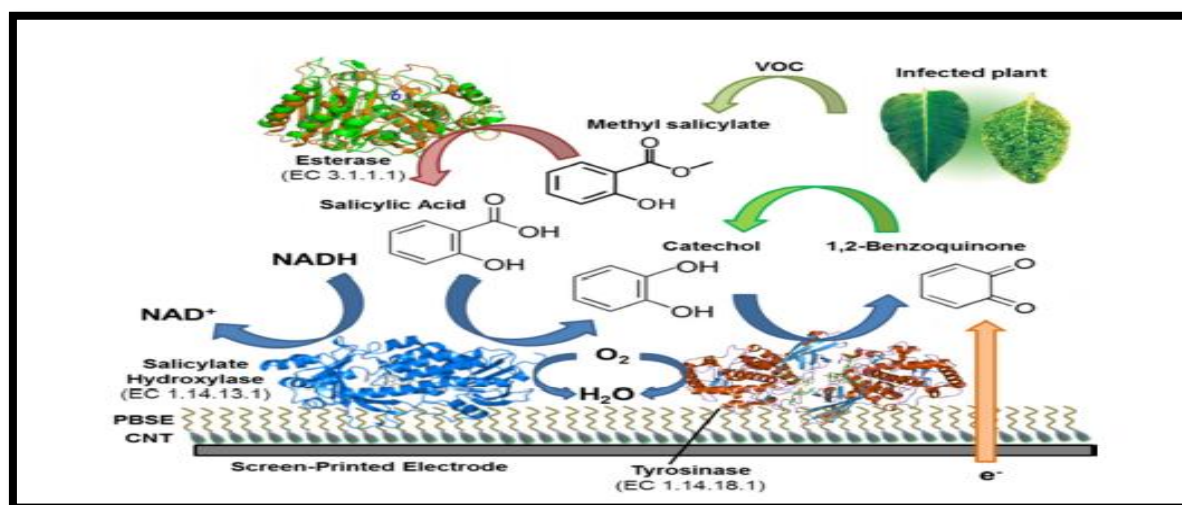


Fig 2. Diagrammatic representation of tri-enzymatic detection of methyl salicylate

Table 1. Detection of VOC using enzymatic biosensor

Volatiles emitted from tomato plant	Enzyme	Pathogen
cis-3-hexen-1-ol	Alcohol dehydrogenase	<i>Botrytis cinerea</i>
Methyl salicylate	Salicylic acid methyl transferase	Tobacco Mosaic Virus

Fang *et al.*, (2015)

Nucleic Acid (DNA/RNA)-Based Affinity Biosensor

This biosensor based on DNA/RNA sequence as biosensing elements for plant pathogen detection. Generally, DNA mediated biosensor can more easily detect the bacterial pathogens as it has distinct nucleic acid sequence, that precisely bind with the complementary DNA sequence. The detection is mainly dependent upon the hydrogen bonds formed between the target DNA sequence and the DNA probe. Other than this, specific DNA hybridization and complementary RNA was also exploited and in way ahead for the detection of various viral diseases in plants with improved Quartz Crystal Microbalance techniques.

Bacteriophage-Based Biosensors

A bacteriophage –based biosensor was developed for detecting and identifying the pathogen *Pseudomonas cannabina* pv. *alisalensis* that causes leaf and stem rot of hemp. In the Fig.3 the *luxAB* (encoding the luciferase) a bacterial reporter gene was integrated into *P. cannabina* pv *alisalensis* phage PBSPCA1. Due to its specific infection through phage the *luxAB* can also be expressed into the host cell. In presence of n-decanal, oxygen and flavin mononucleotide the expressed luciferase results in emission of light which indicate the pathogen infection.(Schofield *et al.*, 2012).

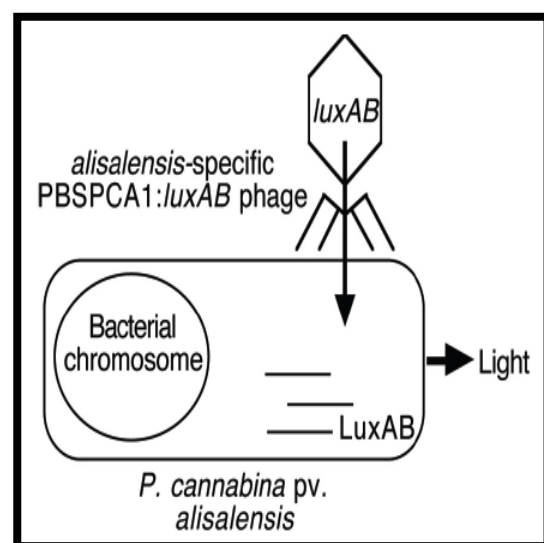


Fig 3. Detection Overview

Application of biosensors in Insect Pest Detection

Dependable pest diagnosis and detection in the early stages of crop plant is relatively perfect to lessen fundamental production and economic losses. The primary purpose of plant pest diagnosis is to evaluate whether or not a plant is healthy and to decide the causes of a disease, if any. However, one foremost task is the problem in determining the physical, chemical, and biological adjustments in plant life for the duration of the

asymptomatic stages of an infection. The primary application of biosensor in insect pest detection are as follows.

1. Early detection of pest
2. Pest population and dynamics
3. Real time monitoring of insects pest

Cantilever nanosensors

The cantilever nanosensor may be used as a organic sensor for early detection of insect pest. A cantilever is a thin, flexible, suspended beam typically constructed from silicon, silicon nitride, silicon dioxide, and polymers. The length and thickness of a cantilever are generally at the order of microns and/or nanometers that provide specific mass, spring constant, resonant frequencies and sensitivities. Cantilever size is very critical because the surface of the cantilever may be covered with a layer which is sensitive to the binding of particular analytes. Cantilever sensors use minimum amounts of power, making them much less expensive to use than conventional sensors. Such biosensors were proved very beneficial in studies of interspecies communication via pheromones.

Micro Electro Mechanical System

MEMS is a pheromone based sensor precise for the interspecies communication of female sex pheromone of lepidopteron pest *Helicoverpa armigera* of cotton, tomato, rice, pigeonpea and chickpea. Silicon dioxide based MEMS devices receive the reaction in terms of change in resonant frequency while the device is exposed to the volatile pheromones. The unique functionalization protocol makes these devices selective and sensitive towards the pheromone. The chemical functionalizations of the devices have photochemical and thermal balance which makes them surroundings pleasant. The functionalized devices may be used at ambient temperature and at different environmental situations, even in wet season or in height summer beneath vibrant sunshine. The maintenance cost for those devices is practically zero and the functionalized gadgets also are reusable due to their engineered reversibility.

The performance of these functionalized devices with the pheromones released by the living *Helicoverpa armigera* pests. The unmated female insects have been separated in pupa level from the male ones, cultured one at a time and 8 of the female insects have been kept in a box covered with a black fabric cloth on top. A continuous flow of air become

maintained from the bottom of the box to direct the pest excreted semiochemicals to the top channel of the container, where the functionalized device had been positioned (Fig. 4). The devices had been taken out after a finite time interval and investigated for their change in the first resonant frequency. Depletion in resonant frequency was found at each time interval of 6, 12 and 24 hr. The change in resonant frequency of the devices in presence of various kairomones released with the aid of tomato seedlings and plant for the duration of the stipulated time durations, which similarly verified the selectivity of the functionalized device toward the pheromones of *Helicoverpa armigera*. The actual area condition became simulated in a huge field containing six tomato vegetation and four of every male and female *Helicoverpa armigera*. The top of the container changed into included through a black material where the female *Helicoverpa armigera* ought to lay eggs. Repeatedly the device showed decrease in resonant frequency establishing the functionalized device as a unique pheromone sensor for *Helicoverpa armigera* (Moitra .P. *et al.*, 2016)

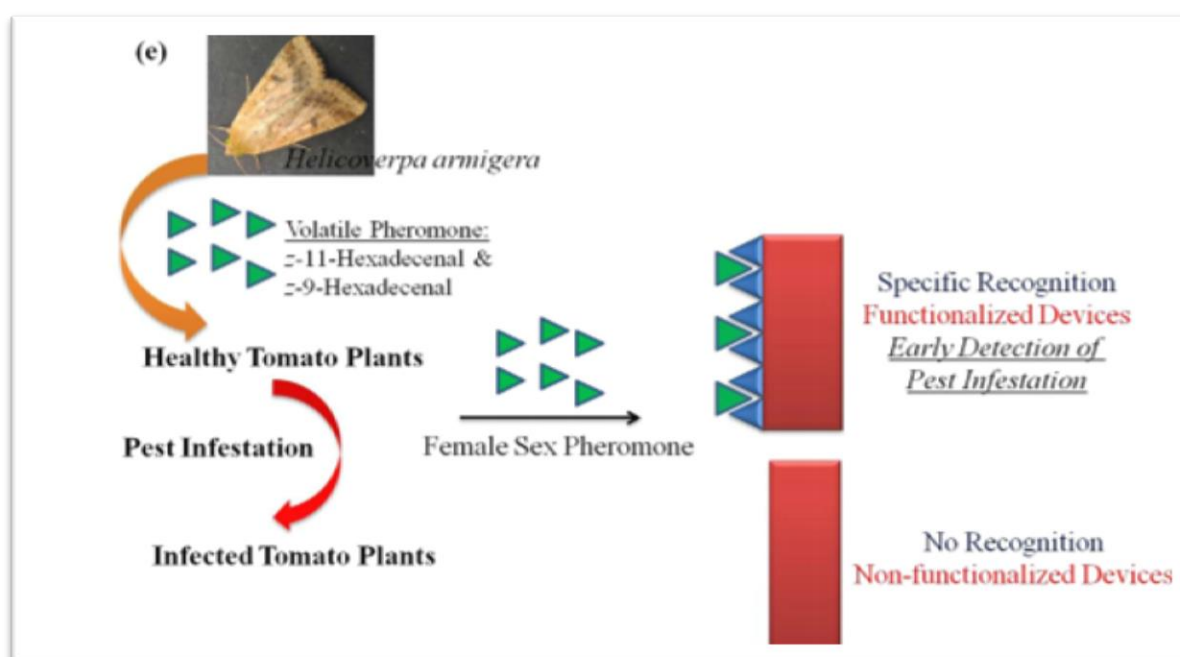


Fig 4. Schematic representation for the detection of female sex pheromone of *Helicoverpa armigera*, *Scirphophaga incertulas*

Plant Pest Detection Using an Artificial Nose System

E-noses, also referred to as artificial olfaction devices, had been extensively applied in numerous application ranging from scientific prognosis to the food industry, environmental safety, and agriculture. These systems are designed to imitate the mammalian olfactory system. They have been coupled with specific forms of sensor arrays, which transform the

VOCs information into an electronic signal. Gas samples when spread across the sensor array, the smell molecules set off reversible physicochemical modifications to the sensing materials. This causes adjustments in electrical properties inclusive of the resistance and electrical potentiality. Conditioning circuits are used to modulate the signals, and pattern recognition is used to classify the aromas. Subsequently, the statistics can be examine, displayed, and saved for pattern recognition evaluation, that may provide primary analysis consequences (Cui Shaoqing *e., al* 2017).

Plant VOCs play important roles in responding to pest attacks and are promising goals for pest-detection. The composition of VOCs emitted by means of vegetation depends on the mode of damage. VOCs are produced via a extensive range of physiological processes in various parts of plant tissues. The defense mechanisms to pathogens or viruses are nonetheless uncertain, however the VOCs had been found to change after plants being infected. Plants require a broad range of protection mechanisms to effectively combat attacks by herbivorous insects or mechanical damage. One of the strategies is to emit unique VOCs to battle potential attacks. While a few protective VOCs are emitted always, others are triggered only in response to herbivore feeding. As a consequence, these variation of VOCs provide authentic principle of pest detection through E-nose.

Spider mites (*Tetranychus urticae*) are herbivorous arachnids that can feed on numerous hundred host plants, which includes economically important tomato plants cultured in greenhouse. E-noses have been used for extensive investigations of VOCs profiles emitted through tomato plants infested with spider mites, under specific growth conditions. The reliability of E-nose technology has been showed in rice plant analysis. Infected rice vegetation attacked by striped rice stem borer (*Chilo suppressalis*) and the brown planthopper (*Nilaparvata lugens*) can be easily discriminated from healthy plant . Moreover, the extent of damage to the rice plant as well as the amount of pests can also be effectively estimated based at the sensor response of an E-nose

Laboratory Sampling

Odor compounds are drawn into E-nose through distinctive series strategies, such as headspace sampling, diffusion techniques, bubblers, and pre-concentrators. Profiling plant vocs are conventionally carried out in a sealed chamber or container with managed temperature and humidity, which simulate the environment of a greenhouse and field.

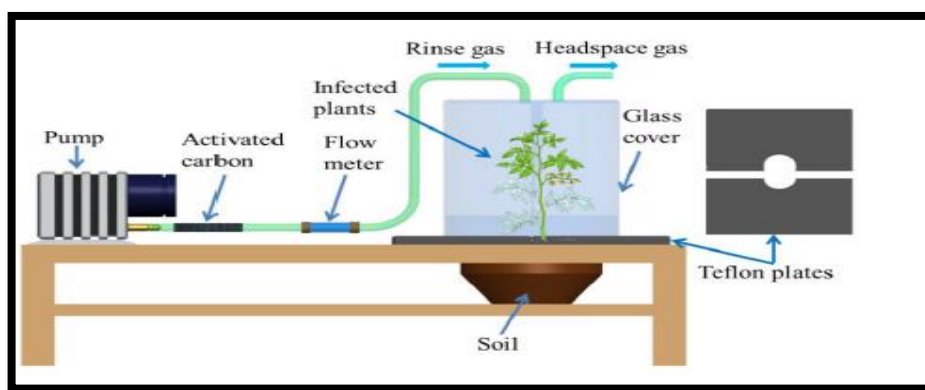


Figure 5. Illustration of collection system for infected plants.

A typical sampling setup is shown in Fig 6. an E-nose was employed to sample the VOCs emitted by powdery mildew and spider mite infected tomato plants, which were housed in clear glass boxes. The humidity and temperature were logged at all times. During the cultivation, clean air was pumped in to create positive pressure to maintain constant environmental parameters and decrease the risk of cross contamination (Zhang *et. al*, 2011)

Conclusion and Way Ahead

Biosensor may solve the problem of screening large numbers of plant disease and pest infecting various fields of economically important crop in a single time. Biosensor technology in general seeks to improve early detection of plant disease and pest by decreasing the analysis time, reducing the pesticides use increasing reliability, and integrating multiple disease detection in a single device. Since, various studies have proved biosensor as a successful scientific device for detection of plant diseases and pest but it still need to be enhanced and modified for it's on field application. Future experiments must be carried how long this biosensor technique can be applicable in food storage field for detection of microbiological infestation.

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Chapter:13

Physiological response to water stress in plant

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ABSTRACT

Crop plant that receive inadequate water experience a stress referred to as water deficit. Plant water deficits disrupt many cellular and whole plant functions, negatively affecting plant growth and reproduction. Plants respond to soil water deficits by means of avoiding the incidence of leaf water deficits (drought avoidance) or tolerating low cellular water contents (drought tolerance). Plant avoids drought by means of closing the stomatal pores that regulating water loss from the leaves, limiting the transpiring area (by depleting growth or dropping leaves) and/or maintaining root water uptake as the soil dries. Plants have evolved many unique mechanisms to tolerate low tissue water contents which include regulation of antioxidant systems to lower the impact of damaging reactive oxygen species. Availability of water is the most essential environmental element that reduces crop production, thus there have been significant efforts by using biotechnologists

Introduction

Drought, a period of abnormally dry weather, results in soil water deficit and subsequently plant–water deficit. Water deficit in the plant disrupts many cellular and whole-plant functions, having a negative impact on plant growth and reproduction. Of the various stresses plants are exposed to (e.g. high temperatures, pests and diseases), lack of water seems responsible for the bulk of crop losses in the field (Boyer, 1982). As water is increasingly needed by expanding human populations and prime agricultural lands are urbanised, water availability will have a greater impact on our ability to produce crops and provide food security for all. Whether the amount of water present in the environment induces a stress is different for each plant species. In nature, certain species are adapted to dry environments and can survive and thrive even in environments with low water availability. Absolute soil water availability, duration of the water-deficit stress, The rate of stress imposition and the developmental stage of the plant when stress occurs also affect plant growth and the ability of crops to produce an economically viable yield. Sensing mechanisms

initiate plant responses to water deficit, which occur at the molecular, metabolic, cellular, physiological and developmental levels. Many of these responses are driven by changes in gene expression.

Plants Can Avoid or Tolerate Water-Deficit Stress

The ability of crops to yield in periodically dry environments can depend on their ability to tolerate water deficits or to develop mechanisms that avoid water deficit (**Figure 1**). Some species, such as desert ephemerals, escape drought by completing their life cycle when water is plentiful. Others avoid water deficit by developing a large root system that extracts water from deep in the soil profile. Water storage in plant tissue is a strategy that succulents (e.g. cactus) employ to avoid water deficit, often in conjunction with mechanisms that reduce the expenditure of water via transpiration. The dominant route of water loss from plants is via pores, which in many plants are predominantly on the underside of leaves, termed stomata. Some plants, such as crassulacean acid metabolism (CAM) plants (e.g. cactus), show modified stomatal behaviour such that stomata are open at night and close during the day. These CAM plants have an alternative form of carbon assimilation that occurs during the night when transpiration is much less than during the day.

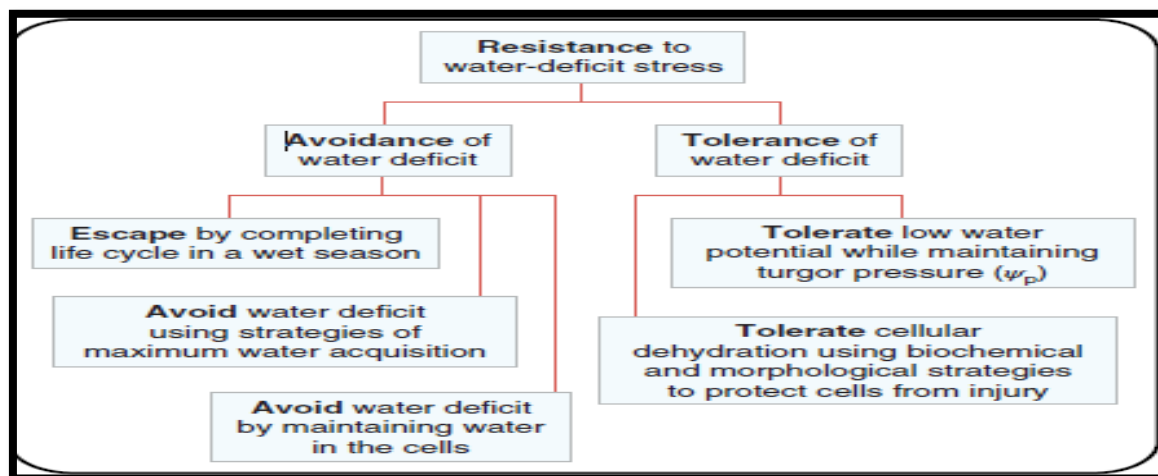


Figure 1. Resistance to water-deficit stress can arise from mechanisms involving avoidance or tolerance of the water deficit

Other plants have biochemical and morphological mechanisms such as that found in mosses and resurrection plants that permit the plants to withstand dehydration and then recover normal function when they are rehydrated. Morphological characteristics of some species permit continued survival in arid environments, while other plants must acclimate to the stress to survive. It is difficult to distinguish which responses are direct results of water-deficit injury and which allow adaptation of the cell(s) to water deficit. Some

physiological mechanisms promote survival and limit injury, but incur a cost of decreased productivity, and are therefore not useful crop improvement strategies. To promote crop production in transiently dry environments, mechanisms that permit continued growth during periods of water deficit should be identified. However, it is important to recognize that any physiological trait can have a positive, negative or neutral effect on crop yield as the soil dries, depending on the dynamics of soil water availability and crop phenology (Tardieu, 2012).

Water Dynamics of the Plant Cell

The water within the cell is defined in terms of its free energy content or ability to do work. The free energy per unit volume of water is the water potential (ψ_w). Water is taken into the plant if the water potential is less than that of the environment surrounding the cell (**Figure 2**), as water moves down a water potential gradient. If the water potential in the soil solution is higher than that of the cells, water can be transported into root cells. Conversely, if the water potential in the soil solution is lower than that of the cells, roots can actually lose water into the soil.

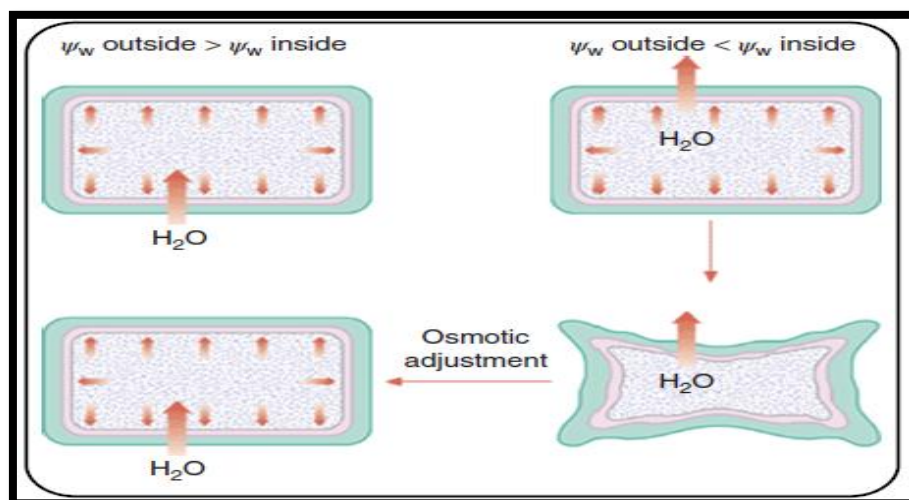


Figure 2: Cellular and soil water potential control water uptake into the cell. Osmotic adjustment, a lowering of cellular osmotic potential caused by solute accumulation/synthesis, can permit water uptake and restore cellular turgor.

For this reason, water exchange between the root and soil is limited to regions close to the root tips, as root maturation results in the laying down of impermeable barriers (composed of suberin, a hydrophobic compound) that prevent water (and solute) exchange.

The water potential of the cell depends on the osmotic potential (ψ_s) and the turgor pressure (ψ_p). Solute content in the water of the cell (ψ_s) and the pressure of the

cellular contents against the cell wall (ψ_p) decrease the water potential. An additional component, the matric potential (ψ_m), or the binding of water to surfaces, also reduces the cell water potential. Equation 1 is used to describe cell water potential, but is usually ignored as it is small in magnitude.

$$\psi_w = \psi_s + \psi_p + \psi_m$$

(1)

Changes in cellular water relations trigger further events that are manifested in plant responses at the molecular, metabolic, cellular, physiological and developmental levels.

Whole-Plant and Physiological Responses

Water within the plant can be viewed as part of a soil–plant–air continuum, with the plant acting as a column of water between the soil and the air. Transpiration (aerial water release from the plant) will continue when water is available and lowers the leaf temperature due to evaporative cooling. As the soil dries (due to plant water uptake), the remaining water in the soil is held more tightly by matric forces (thus decreasing soil water potential), which can restrict water movement into the root cells. Unless the plant regulates its water loss (see the following discussion on stomatal closure), water lost through transpiration may not be fully replaced, decreasing turgor in the plant cells. Maintaining turgor is necessary for the plant to retain its structure, allow continued growth and prevent damage to cellular metabolism. To maintain whole-plant turgor, the pore of the stomatal complex closes in response to soil water deficit. As turgor decreases in the guard cells surrounding the stomatal pore, the cells fill the pore, thus reducing the stomatal aperture; thereby reducing transpiration. Guard cell turgor can decrease in response to cellular dehydration (a direct effect of drought) or in response to intercellular signals (e.g. plant hormones) that stimulate solute loss from the guard cells. Solute loss from the guard cells to the apoplast (the cellular compartment surrounding the guard cells) will be accompanied by water loss via diffusion, thereby decreasing guard cell turgor. While stomatal closure is essential to minimise water loss, carbon dioxide uptake is also restricted and can limit photosynthesis. Thus, there is considerable interest in improving leaf transpiration efficiency (defined as the ratio of photosynthesis to transpiration) which can be correlated with crop water use efficiency (defined as the ratio of whole-plant biomass to water use).

An *Arabidopsis* leucine-rich repeat receptor-like kinase (ERECTA) regulates leaf transpiration efficiency by affecting both stomatal density and photosynthetic capacity (Masle

et al., 2005). There is also considerable interest from both plant breeders and biotechnologists alike in improving transpiration efficiency in major crop plants (Condon *et al.*, 2004), but selecting plants for high transpiration efficiency may not always result in higher yields in droughted environments. Cellular expansion or growth depends on cellular turgor and will be inhibited as turgor approaches zero. The pressure of the cellular contents against the cell wall drives cell expansion and turgor depends on the continued uptake of water. In monocotyledons

(such as wheat and maize), increased xylem tension caused by high rates of transpiration can reverse the normal water potential gradient between the xylem and growing cells, interrupting cell expansion. Growth will return if xylem tension decreases, and as turgor is restored as the plant acclimatises to water-deficit stress.

Adaptation to Water Deficits: Osmotic Adjustment and Aquaporins

In a process called osmotic adjustment, cellular metabolism is altered to increase cellular solute concentrations, thereby maintaining cellular water content via diffusion. In these cells, the osmotic potential is lowered and thus the water potential of the cell is lowered, thereby maintaining water uptake (**Figure 2**). Turgor will fully or partially recover depending on the external water potential. This active process is distinct from any passive increase of solute concentration that occurs due to cellular dehydration. There is considerable genotypic variation in the ability to adjust osmotically, and this variation has been exploited in some crop improvement programmes. Nevertheless, there is no consistent relationship between osmotic adjustment and crop yields, with positive relationships reported only in environments with severe

water deficits (Serraj and Sinclair, 2002), likely due to the minimal benefits of diverting assimilates to osmolyte production (with associated energetic costs) under mild–moderate water deficits. The solutes that accumulate, called osmolytes, include sugars, proline and quaternary ammonium compounds such as glycinebetaine. They are generally regarded as neutral to metabolic processes and therefore do not disrupt plant function. Many studies have genetically engineered plants to accumulate either these natural osmolytes or osmolytes that would not normally occur within the plant. For example, plants engineered to produce more glycinebetaine have improved photosynthetic capacity when grown under water-deficit conditions, even when the plants do not naturally produce glycinebetaine (Wani *et al.*, 2013). Interestingly, osmolyte accumulation is often insufficient to substantially alter osmotic adjustment, yet plant performance in drying soil is improved nonetheless. Thus, osmolytes

may enhance drought tolerance by stabilising proteins and ameliorating oxidative stress that may arise when plants are subjected to water deficit. These overexpression studies may indicate that these alternative roles, rather changes in osmotic adjustment, are more important in mitigating the effects of drought. Membrane permeability to water and ions is also involved in the control of cellular water potential and turgor. Aquaporins are proteins that form a channel in the membrane that facilitates

transport of water (and other small solutes) across the membrane using an osmotic or hydraulic driving force. Plasma membrane intrinsic (PIP) and tonoplast intrinsic (TIP) are water channels in the plasma membrane and tonoplast, respectively. Water deficit often decreases transcript abundance of PIP genes, which may limit loss of water from the cells. Studies that have upregulated aquaporin expression suggest that these proteins regulate root hydraulic conductivity, water-deficit stress resistance and the ability of plants to recover from water-deficit stress. The severity of water deficit likely determines the benefits of changing aquaporin expression: upregulating aquaporin overexpression may be beneficial under mild–moderate water deficits when root hydraulic conductivity limits water uptake more than soil hydraulic conductivity, while downregulating aquaporin overexpression may be beneficial under severe water deficits to prevent water loss to the soil.

Photosynthetic Responses and Antioxidant Systems under Drought

When the stomata are closed, uptake of carbon dioxide is also reduced, reducing the carbon assimilation rate of the plant. Depending on the duration of the water deficit, this may reduce crop production and cause injury to the chloroplasts through the process of photoinhibition. (Photoinhibition is defined as any light-induced decrease in the efficiency of photosynthesis.) Usually, drought per se has limited effects on photoinhibition unless cellular turgor is very low, but drought may interact with other stresses, such as heat stress, when transpiration is reduced, to synergistically inhibit photosynthesis. Plant drought tolerance can be improved and yield maintained by enhancing the antioxidant system. Drought reduces the content, activity and regeneration of key enzymes involved in the photosynthetic carbon cycle. These metabolic impairments reduce the biochemical capacity to assimilate and use carbon. Together with stomatal closure and increased leaf temperature (which can lead to membrane denaturation), these limitations to carbon assimilation cause a disparity between the number of photons absorbed by the light-harvesting complexes and the amount used for photochemistry. This decreases the efficiency with which the photosynthetic apparatus can work and results in a build up of excess energy and electrons which must be

dissipated. While a number of strategies may be employed to limit the damage, some (such as the formation of triplet states of chlorophyll) may form damaging reactive oxygen species (ROS). While some increase in ROS is necessary to optimise cell functions and activate appropriate responses such as stomatal closure, under drought foliar ROS content and lipid peroxidation can significantly increase when compared to well-watered plants (Begcy *et al.*, 2011). This may permanently damage the photosynthetic apparatus, ultimately reducing crop yield and quality (Miller *et al.*, 2010).

The Role of the Signalling Molecule Absciscic Acid

Cellular loss of water is a physical stress that initiates biochemical events. Cellular mechanism(s) are thought to recognise a decrease in water content, probably related to turgor pressure, and induce subsequent signal transduction pathways. One intermediate signaling molecule is the plant hormone absciscic acid (ABA). Plant ABA concentrations increase in response to water deficit due to alterations in ABA biosynthesis and catabolism. Depending on the speed at which water deficits are imposed, experiments have demonstrated that root ABA concentration can increase before leaf ABA concentration or *vice versa*. Ultimately, ABA accumulates in all plant organs and this response is important for physiological and molecular responses to water deficit, with the most studied response being stomatal closure. Unravelling the ABA biosynthesis pathway required a number of genetic, biochemical and molecular approaches. Carotenoid breakdown, rather than synthesis from a smaller carbon backbone, occurs. During periods of water deficit, the enzyme, 9-*cis*-epoxycarotenoid dioxygenase (encoded by a family of genes named *NCED*, with nine members in *Arabidopsis*), completes the oxidative cleavage of *cis*-xanthophylls to xanthoxin (**Figure 3**). There are two steps remaining in the synthesis of ABA with ABA-aldehyde as the immediate precursor to ABA. The gene *ABA2* from *Arabidopsis* encodes a short-chain alcohol dehydrogenase that converts xanthoxin into absciscic aldehyde. Absciscic aldehyde oxidase 3, *AAO3*, one of four such oxidases in *Arabidopsis*, oxidises absciscic aldehyde to ABA. This enzyme requires a sulfurylated form of molybdenum cofactor (MoCo), and thus the *Arabidopsis* gene *ABA3* (a MoCo sulfurase) is also required for ABA biosynthesis. In response to water-deficit conditions, *NCED3*, *AAO3* and *ABA3* are all induced.

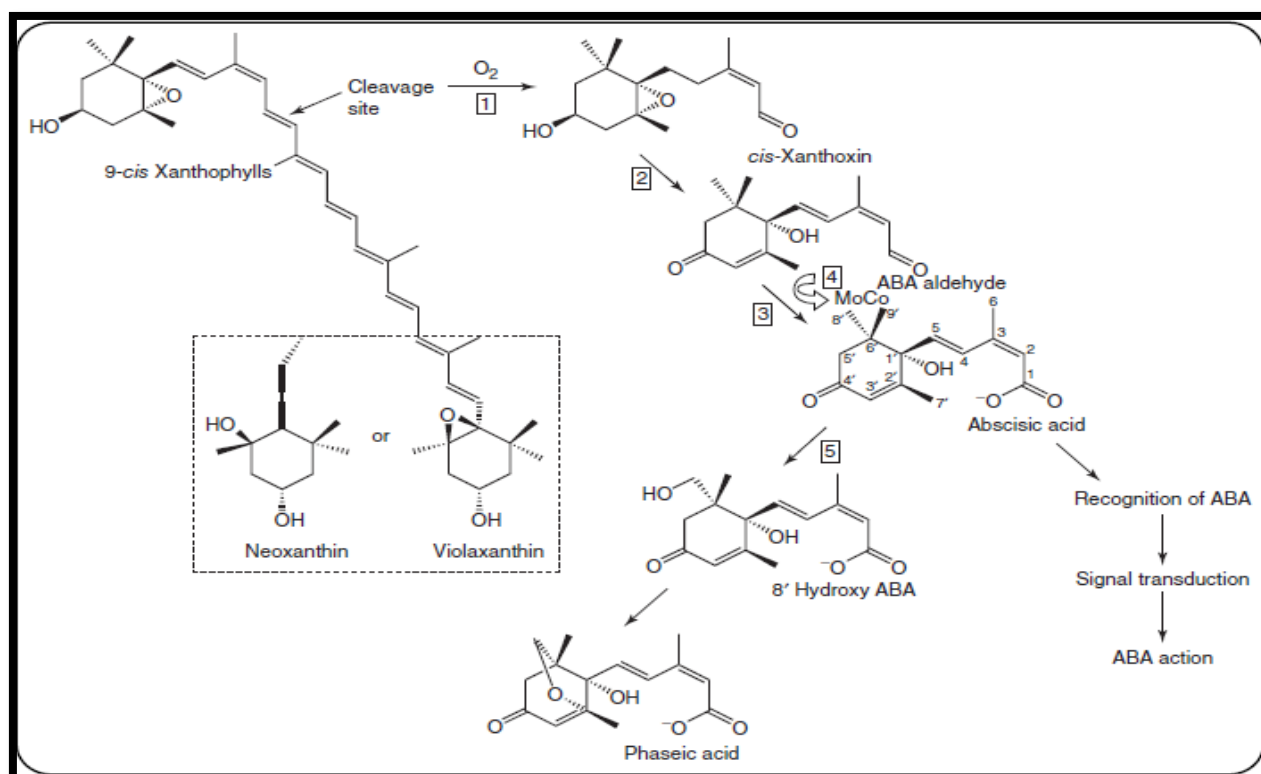


Figure 3: ABA biosynthesis beginning with a carotenoid and proceeding through the major pathway for catabolism. Both synthesis and breakdown contribute to the level of ABA in a particular organ of the plant in response to water deficit. The numbers in boxes represent enzymes that have been cloned in *Arabidopsis*. (1) 9-cis-epoxycarotenoids dioxygenase 3 (NCED3) catalyses the cleavage of *cis*-xanthophylls during water-deficit stress. (2) The product xanthoxin is converted into ABA aldehyde by a short-chain alcohol dehydrogenase, ABA2. (3) Absciscic aldehyde oxidase AAO3, an enzyme that requires a sulfurylated form of MoCo (synthesised by (4) MoCo sulfurase (ABA3)), completes the final step of ABA biosynthesis. (5) The key step of ABA catabolism is the hydroxylation of the 8' methyl group to yield 8' hydroxy ABA by a cytochrome P450 monooxygenase CYP707A3. Phaseic acid is then formed spontaneously. Increased ABA concentrations initiate a signal transduction pathway, leading to the induction of specific genes.

ABA breakdown also has an important role in controlling plant ABA concentrations. ABA is catabolised by hydroxylation and conjugation (**Figure 3**). The hydroxylation pathway results in oxidation of the 7', 8' or 9'-carbon of the ring structure. The major pathway of catabolism begins with 8' hydroxylation by the cytochrome P450 monooxygenase CYP707A, followed by spontaneous conversion to phaseic acid. Dihydrophaseic acid (DPA) and DPA glucoside are further catabolites. ABA and its hydroxylated catabolites may also be conjugated with glucose, forming a storage form which may be liberated by the action of β -glucosidase enzymes (Lee *et al.*, 2006). ABA accumulation initiates a series of events (**Figure 3**) which promote plant adaptation to water

deficit. Firstly, ABA must be recognised in the cell. There has been considerable recent progress in identifying ABA receptors (Miyakawa *et al.*, 2013). ABA recognition initiates a signal transduction pathway that is composed of such signalling components as kinase/phosphatase cascades, RNA (ribonucleic acid)-processing proteins and calcium. Mutants in ABA signalling pathways are being used to unravel the complex interactions that control ABA responses. At a physiological level, ABA appears to play important roles in regulating stomatal closure, root growth and function and possibly the regulation of leaf growth (Tardieu *et al.*, 2010). Furthermore, advances in our understanding of ABA signalling (and other phytohormones) and other signalling pathways are now being exploited by biotechnologists to create transgenic plants and the development of irrigation strategies.

Root Mechanisms to Enhance Water Uptake

Mild soil drying can actually stimulate root growth, and alternate wetting and drying of the soil can dramatically increase root biomass and lateral root development. Although the expansive growth of leaves (and other shoot organs) is highly sensitive to decreased tissue water potential, roots can continue to grow at water potentials that inhibit the growth of other organs. As the soil dries, these physiological and morphological adaptations may allow continued water uptake. Roots exposed to drying soil accumulate high concentrations of the phytohormone ABA, although there is some debate as to whether the roots sense local changes in soil matric potential or respond to an integrated measurement of root water potential. The role of ABA in regulating growth of roots in dry soil has received considerable attention. When the soil is moist, ABA accumulation seems to inhibit root growth. When the soil is dry, ABA accumulation is essential to limit the synthesis of ethylene, another phytohormone that inhibits growth (Spollen *et al.*, 2000). Furthermore, root ABA accumulation appears necessary for the synthesis of osmolytes such as proline to maintain continued water uptake into the root.

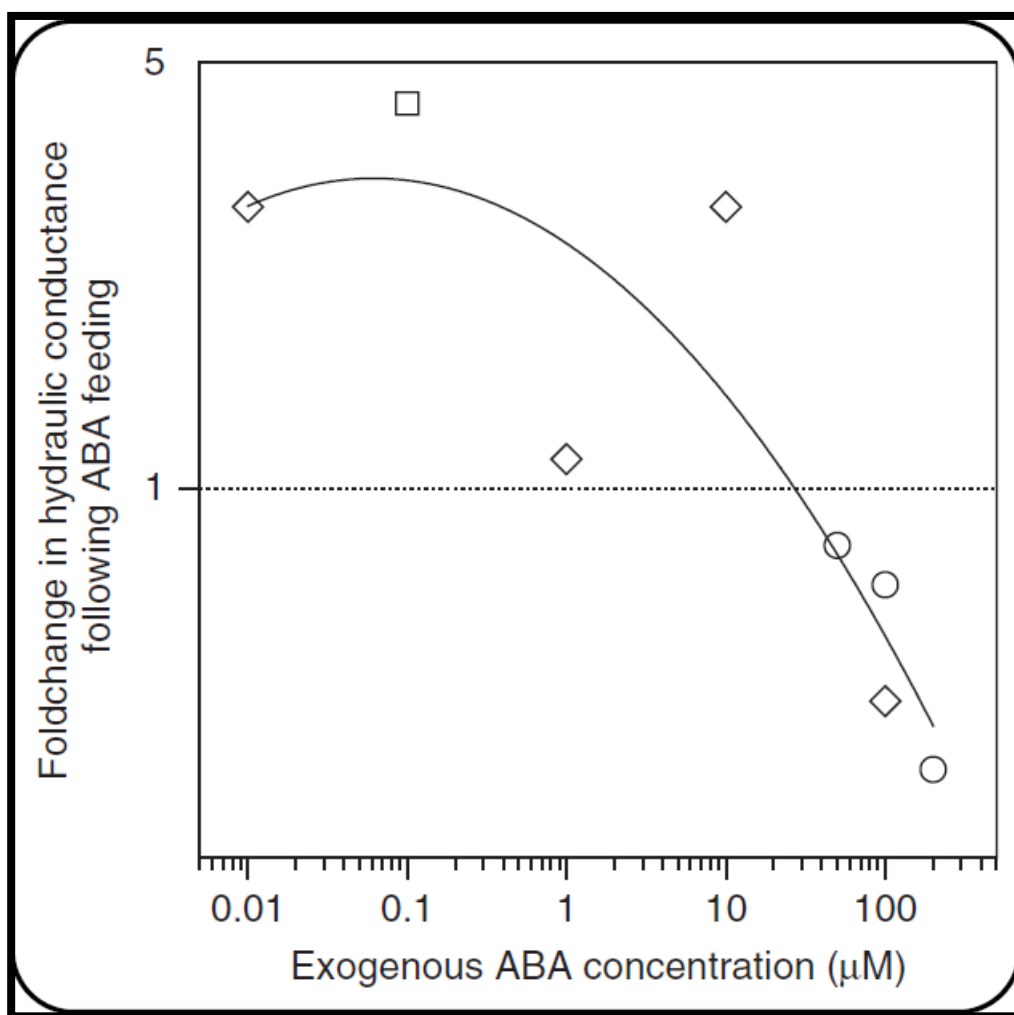


Figure 4 Fold change in root system hydraulic conductance in response to nutrient solution-supplied ABA.

CONCLUSION AND FUTURE PERSPECTIVE

Due to the sessile life cycle, plants have evolved mechanisms to respond and adapt to adverse environmental stresses during their development and growth. Plant growth is impaired by severe drought stress due to a decrease in stomatal opening, which limits CO₂ uptake and hence reduces photosynthetic activity. In order to develop strategies to maintain plant productivity, it is essential to understand the various regulatory mechanisms that control and enhance adaptive responses to stress in different plant species.

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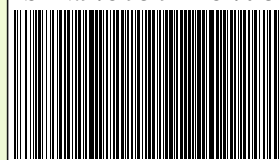
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