INSTITUTION OF AGRICULTURAL TECHNOLOGISTS,

BENGALURU

EVALUATION OF RKVY PROJECTS

OF

UNIVERSITY OF AGRICULTURAL SCIENCES,

RAICHUR

"HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES"

INSTITUTION OF AGRICULTURAL TECHNOLOGISTS,

#15, QUEENS ROAD, BENGALURU 560 052

CONTENTS

Sl. No.	Particulars	Page No.
1	Executive Summary	1
2	Introduction	10
3	Objectives	25
4	Hypothesis	25
5	Objectives and issues for evaluation	27
6	Stake holders and purpose of evaluation	27
7	Evaluation Design	27
8	Data collection and analysis	29
9	Findings and discussion	33
10	Utilization of funds	39
11	Reflections and conclusions	41
12	Recommendations	44
13	References	46
14	Terms of Reference	56
15	Evaluation Team Members	62

HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES

EXECUTIVE SUMMARY

Insects are vital to the overall order of many ecosystems and have lived collectively with the human population for centuries. While insects are viewed as beneficial in several facets of society, they also endanger the human population. As agricultural pests, insect species have been the cause of food source damage and depletion, resulting in substantial economic losses. Progress in crop protection by chemical has been extraordinary over the last decades, not only in the invention of new and selective active ingredients but also in the assessment of the behavior of these chemicals in the environment.

Promising control results with most insecticides, however, have been short-lived, with the development of insecticide resistance, the culmination of factors including pesticide misuse, lack of novel compounds in the pipeline, and a dearth of diversity in the mode of action. Insecticide resistance has been reported in areas worldwide, with the most commonly used compounds such as synthetic pyrethroids, organophosphates, and chlorinated hydrocarbons, being less effective in targeting and altering the insect nervous system (WHO 2014).

Insecticide resistance, therefore, has been a contributing factor to continued persistence of agricultural pests and the resurgence of vector-borne diseases, highlighting the importance of (i) identifying and developing insecticides with alternative modes of action and (ii) alternative control approaches.

Intensive agriculture, which is associated with heavy inputs of synthetic insecticides, has serious ecological impacts, leading to loss of vital ecosystem services including insect-mediated pest suppression. In recent years, efforts have been made towards obtaining safer options to chemical insecticides for sustainable pest management.

The use of plant extracts as botanical insecticides is also an important provisioning ecosystem service. Integrated pest management (IPM) is an example of redesigning intensive agricultural systems. Instead of relying principally on synthetic pesticides, IPM uses non-chemical or botanical insecticide measures to suppress pest population increase and a range of curative management tactics with synthetic pesticide use as last resort (Barzman et al. 2015). The declining availability of many pesticides due to resistance and deregistration, reflecting increasing awareness of their environmental and human health

consequences, has driven changes towards ecologically based practices (Barzman et al. 2015; Borel 2017; Chagnon et al. 2015; Li et al. 2017; Sumon et al. 2018).

Ecologically based pest management tactics such as conservation biological control have been shown to reduce the use of synthetic insecticides in a variety of cropping systems whilst maintaining or increasing crop yields and efforts are being made to up scale the practice globally (Pretty et al. 2018; Wyckhuys et al. 2013; Xu et al. 2017). Despite these advantages, however, uptake of conservation biological control on a wide scale is limited (Gurr et al. 2016). In cases where uptake has been strong, the vegetation used in habitat manipulation provides multiple ecosystem services rather than suppressing pests alone (Khan et al. 2006, 2012). To date, however, there is a major gap in knowledge about the possibility of habitat manipulation plants providing botanical insecticides. This is important because synthetic insecticides present significant risks to human health. Agricultural workers and consumers are at risk of being negatively affected by insecticide products, tank mixes, drift, residues and breakdown products, especially as a consequence of poor registration, storage and misuse (Eddleston et al. 2002). In agricultural areas where there are high illiteracy rates, and poor training and equipment, the impacts are especially high (Amoabeng et al. 2017; Williamson et al. 2008).

Many plants possess secondary metabolites such as alkaloids, phenols and terpenoids that can have insecticidal activity such as toxicity, repellency, feeding deterrence against insect pests (Koul 2004). Botanical insecticides, including extracts and essential oils of these plant species, have been used to protect crops against insect herbivory for many years (Belmain et al. 2012; Isman 2000, 2008). Synthetic insecticides often have lethal and sub-lethal effects on natural enemies (Desneux et al. 2007). Biopesticides are considered relatively benign to non-target species owing to their rapid breakdown, selectivity nature and reduced risk of insecticide resistance as plant extract, particularly crude extracts have multiple modes of action other than toxicity, such as repellency (Amoabeng et al. 2013; Dubey et al. 2011; Isman 2006; Koul et al. 2008; Tembo et al. 2018). Another important benefit of botanicals is that they tend to depend on “suites” of closely related active constituents rather than a single active ingredient; this diversity may delay or mitigate the development of resistance in pest populations to most botanicals (Koul 2004). Biopesticides have been used for centuries as means of managing pests until synthetic insecticides replaced plant extracts (Isman 1997). The interest in botanical insecticides is increasing but still accounts for less than 1% of crop protectants used globally (Isman 2008, 2017). In developing countries, plant extracts are often prepared from common weed species that grow around the field and obtained freely, with labour as the only cost, resulting in cheaper pest management option when compared with synthetic insecticides (Amoabeng et al. 2014; Isman 2017).

Highthroughput screening

Highthroughput screening is a method of scientific experimentation that comprises the screening of large compound libraries for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis. High throughput is an adjective use before screening to become fastest - first and best. It represents high speed of screening throughout process and reflects what chemists can easily work upon. Use of *in-vitro* and *in vivo* assays against molecular targets for the evaluation of chemicals as lead structures in pesticide discovery (Duke J., and M. Bogenschutz, 2002). Traditional *in vivo* screening processes are time consuming, laborious, slow in process, requires larger space. *In vivo* high throughput screening permits rapidly screen large amounts of compounds for biological activity. The natural substance extracts are then automatically tested by ultra-high-throughput *in vivo* screening (UHTVS), which is performed for appropriate insecticidal, fungicidal and herbicidal efficacy. If the robots discover that a sample has the desired biological effect, it goes through further stages of isolation before being tested for its suitability as a possible pesticide (Shaon kumar Das, 2016).

Plant parasitic nematodes are harmful plant pathogens causing much more damage annually compared to insect pests, they cause projected yield loss of 12.3% ($157 billion dollars) worldwide. Out of which $40.3 million is reported from India (Singh et all., 2015). Farmers/growers identified insect pests, and other constraints as production problems but overlooked plant parasitic nematodes. Plant parasitic nematodes (PPNs) are causing serious yield loss in a wide range of plants and agriculture crops (Perry and Moens, 2011). Nematode diseases are difficult to control because of their hidden nature and hence, more often overlooked. Plant parasitic nematodes not only cause damage individually but form disease-complexes with other micro-organism and increased the crop loss.

Fungal diseases in developing countries demand special attention. The general impact of fungal pathogens on human health goes beyond the ability of fungi to infect humans, since they destroy a third of all food crops annually (Fisher et al., 2012), causing economical loss and impacting global poverty. Statistics from the 2009–2010 world harvest (www.fao.org or FAOSTAT1) suggest fungi-induced losses in five of the most important crops globally (rice, wheat, maize, potatoes, and soybean). If those losses were mitigated, these crops would have been enough to feed 8.5% of the seven billion populations in 2011 (Fisher et al., 2012). The most economically devastating fungi are *Magnaporthe oryzae*, affecting rice and wheat, followed by *Botrytis cinerea*, which has a broad host range and Puccinia spp., affecting wheat (Dean et al., 2012). Several high-value crops produced in the tropics, such as bananas, coffee, cacao, spices, mangos, and several nuts, are currently

affected by fungal infections and these crops are not produced colder climates (Drenth and Guest, 2016).

Most of the crop plants are attacked by seed and soil borne diseases. Among those pathogenic fungi, Aspergillus flavus and Rhizoctonia bataticola are known to infect and causing heavy losses. The disease severity depends upon the temperature and moisture conditions. For the management of Aspergillus flavus and Rhizoctonia bataticola synthetic fungicides are known to be effective. However, the use of synthetic fungicides is limited by the emergence of resistant fungus strains and some fungicides possess considerable toxicity. Moreover, there is a growing public concern over the increased health and environmental hazards associated with synthetic molecules. While the scientific development of new insecticides has plateaued, great strides have been made in the field of insect genomics. Emphasis is given now to develop extracts from essential oils and plants for the management of Aspergillus flavus and Rhizoctonia bataticola.

Antifungal investigations have revealed that plant extracts like garlic extract, some essential oils from *Mentha spicata* L., *Foeniculum miller*, *Azadirachta indica*, *Conium maculatum* and *Artemisia dracunculus*, active ingredients of cinnamon like cinnamaldehyde and eugenol, different plant extracts like neem (*Azadirachta indica*) seed kernel extract (NSKE), Pongamia (*Pongamia pinnata*) oil and nimbicidin, aqueous extract of *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopisjuliflora, Punica granatum* and *Sygigium cumini* have very good antifungal activity against one or the other Aspergillus species tested.

Similarly, antifungal activity of aqueous extract and oil of neem (*Azadirachta indica*), leaves of *Allamanda cathartics* and *Artabotrys hexapetalus*, ajowain (*Trachispermum ammi*), lemon grass (*Cymbopogon citratus*), Tulsi (*Ocimum sp.*), mentha (*Mentha sp*.), *Rauwolfia sp*., mehandi (*Lawsonia inermins*), and samhalu (*Vertex trifolia*), 24 botanicals belonging to the family Compositae have been well researched and established.

Keeping the above in view, the project, "**HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES"** was taken up by Department of Molecular Biology and Agricultural Biotechnology, University of Agricultural Sciences, Raichur with Rashtriya Krishi Vikas Yojana funding. The project was implemented during 2016-17. The details of the project are as under:

1.	**Title of Project**	**:**	**"HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/ NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES"**
2.	**Nodal officer and Principal Investigator**	**:**	**Dr. B. Kisan,** Asst. Professor and Head, Department of Molecular Biology and Agricultural Biotechnology, College of Agriculture, University of Agricultural Sciences, Raichur
3.	**Implementing Institution (S) and other collaborating Institution (s)**	**:**	Department of Molecular Biology and Agricultural Biotechnology, University of Agricultural Sciences, Raichur
4.	**Date of commencement of Project**	**:**	2016-17
5.	**Approved date of completion**	**:**	2016-17
6.	**Actual date of completion**	**:**	2016-17
7.	**Project cost**	**:**	Rs. 30 lakhs

The objectives of the project are as follows:

1. Obtaining chemical scaffolds/ molecules for screening.
2. Developing highthroughput screening platform.
3. Testing the molecules for their effectiveness.
4. Evaluation of efficacy on major insect/ pests.

The focus of Evaluation is:

i. To examine whether the screening procedure based on 96 well plate and utility of imaging and other methodologies adopted was able to expand the screening procedure faster, cheaper and in timely fashion.

ii. To evaluate plant extracts, essential oils and synthetic chemical compounds to screen for their activity against the nematodes and fungal pathogen inhibition.

iii. To evaluate the efficacy of molecules selected on control of diseases

The intention of the scheme is to develop a screening procedure using sophisticated systems and assays that can evaluate plant extracts, essential oils and synthetic chemicals for their activity against nematodes and fungal pathogens and evaluating the efficacy of the molecules for control of diseases in the field. The underlying logic is;

a. Screening of large compound libraries for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis becomes fastest - first and best.
b. It represents high speed of screening throughput process and reflects what chemists can easily work upon.
c. Emphasis on use of plant extracts and essential oils in place of synthetic chemical compounds to screen for their activity against the nematodes and fungal pathogen inhibition will pave the way for development of pest management systems that minimize health and environmental hazards.

The research work was carried out during 2017-18 at the Department of Molecular Biology and Agricultural Biotechnology, College of Agriculture and Main Agricultural Research Station, University of Agricultural Sciences Raichur, Karnataka (India).

The identified pure culture of pathogenic fungi Aspergillus flavus and Rhizoctonia bataticola were collected from Department of Plant Pathology, College of Agriculture, UAS, Raichur for the study. The Aspergillus flavus and Rhizoctonia bataticola cultures were subcultured on PDA slants and kept at 28 ± 2 ºC for 7 days. Those slants were preserved in refrigerator at 4 ºC and maintained by sub-culturing once in a month in order to avoid a decline in strain viability. Such cultures were used throughout the study.

Twelve essential oils, 24 plants extracts and 64 synthetic chemicals were screened using microtiter plate method, food poison method, pyocynin assay and further confirmed by the Resazurin assay for activity against the pathogenic fungi Aspergillus flavus and Rhizoctonia bataticola.

FINDINGS AND DISCUSSION

Among the essential oils (EOs) tested, OL-2 and OL-12 combination of EOs for concentrations 0.25, 0.5, 1 and 2 per cent, cent percent mortality observed at time intervals 10 min onwards. The essential oils independently brought about 100% mortality within 12 hours of incubation.

Among the essential oil extracts screened with Food Poison Technique, inhibition of mycelial growth was found be significant with orange oil and cinnamon oil.

The results of screening a few essential oils showed that they inhibited the nematode In-vitro at 1% concentration on exposure within an hour of incubation and further the combination of different essential oils provided the effective control within 30

minutes of exposure and these tests were confirmed with model system *Caenorhabditis elegans* and further with M*eloidogyne incognetta*, the root knot causing nematode. The studies indicated that control of the nematode is possible by further formulating these essential oils and testing in pot and field conditions.

Further, studies are required to bring out the best combination of synthetic and organic based formulation to control the nematode population in the field as the seven chemical compounds, two essential oils showed the effectiveness in controlling nematodes at 10ppm concentrations.

The studies will help the farming community by finding new solutions to control the disease causing nematodes and avoid loss of crop. It will also help in floriculture sector, horticultural crops and fruit crops by controlling the nematode infestation resulting in better yields and profit to farmers.

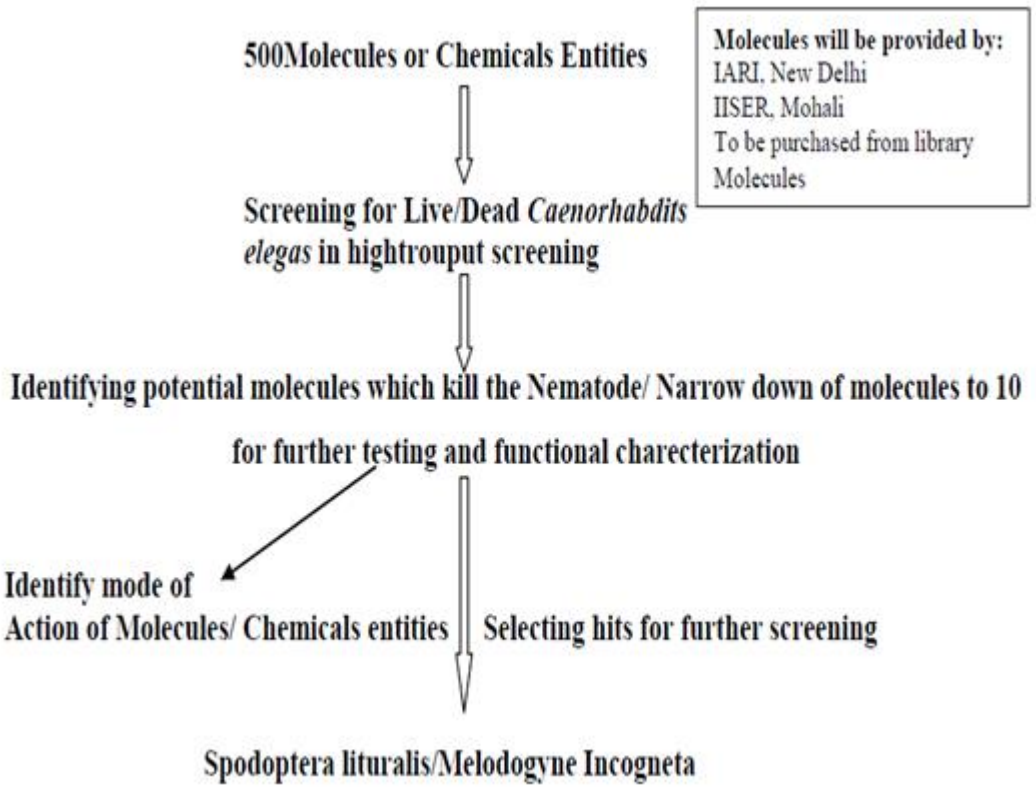
The screening for the fungicidal activity with essential oil was evaluated by microtiter method, food poison method, pyocynin assay and further confirmed by the Resazurin assay for controlling cell disruption there by controlling the fungal inoculum. Further testing in pot culture and field evaluation needs to be carried out.

Efficacy in field studies:

With good results in *in vitro* studies, experiments two formulations with the concentrations ranging from 0.5 to 10% were done by spraying in a small scale field trial to evaluate their performance in Chilli and Ber. During the study phytotoxicity was observed above 5% concentration of the spray. It was found that the formulations were able to control the powdery mildew of Ber and Chilli to the tune of 70%. These formulations are organic in nature. Hence, ease on environment, environment friendly, farmer friendly, do not cause any pollution and resistance development will not be possible as these essential oil and formulations will be having more than 30 compounds which will be acting simultaneously. Hence, longer utility and profitability to the farmers and farming community can be assured. This formulation is under field test in second year of evaluation on various vegetables and fruit crops. This formulation will boost the farmer's economy and saves the crop from loss on account of powdery mildew which is a major disease in chilli and other vegetables. This formulation also increases productivity by enhancing, retaining the chlorophyll for longer periods as the crop is protected from the powdery mildew and the fruit bearing capacity and size of the fruit is enhanced in these sprays.

During the implementation of the project, it has been possible to standardize the screening protocol with the help of Highthroughput screening using *Caenorhabdits elegans* as model nematode. Potential molecules which control the nematodes have been identified

and narrowed down for further testing and functional characterization. Identifying the mode of action of molecules and selecting hits for further screening will have to be carried out.



ACTION POINTS

1. The project has been well conceived and systematically executed and has been successful in examining whether the screening procedure based on 96 well plate and utility of imaging and other methodologies adopted is able to make the screening procedure faster, cheaper and in timely fashion. This will help in screening molecules for action against insect pests and diseases.
2. As a future step arising out of the project, identifying the mode of action of molecules and selecting hits for further screening will have to be carried out.
3. Imaging of *Caenorhabdits elegans* using SPIM, Light Sheet Microscopy or Spinning Disk Confocal with Zyla 4.2 Plus and Sona sCMOS cameras has helped in high resolution imaging performance and Sona is the most sensitive sCMOS camera with a very large field of view, superior longevity and quantitative accuracy.
4. Molecular targets need to be established
5. Testing formulations to control fungal and nematode in pot cultures and field conditions needs to be carried forward for *Aspergillus flavus, Fusarium oxysporum*

uduum and *Rhizoctonia bataticola.* No clear description about major achievemets on anti parasitic molecule / extracts from highthroughput screening have emerged. The report highlighted that, very good antifungal activity against one or the other Aspergillus species tested. But to find utility of the above, more quantitative results are required. Otherwise, extending the same to practical application is difficult.

6. While several essential oils and plant extracts have been evaluated for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis, there is need to identify the molecules responsible for effective control, mode of action and comparative costs to study the economics of disease management. The plant extracts obtained from Jain University is not even partially characterized. Further, the synthetic chemicals from Kuvempu university is not explained in detail about the chemistry and properties. The report is not providing more comprehensive quantitative result of potential individual essential oil/extract/synthetic chemicals with reference to any of the leading reference standards.
7. Specific role of the essential oils, plant extracts and synthetic chemicals are not well presented. Therefore, it is very difficult to draw any specific conclusion about the beneficial role of above chemicals, extracts, synthetic chemicals in comparison with a reference standard and their practical utility.
8. The infrastructural facilities created at the centre was used by three masters students for their research work. Two projects have been received from Department of Science and Technology, Government of India which will be taken up by using the facilities created by the project.
9. The facilities are also being used by students from various disciplines such as nematology, dental pathology, microbiology and biotechnology from different institutes. It is recommended that the facilities should be exclusively used for the development of agriculture by taking up more research in the field. If at all the facilities are spared, it should be on revenue generation method since the techniques involve expenditure.

RESEARCHABLE ISSUES

1. Relevance of secondary metabolites such as alkaloids, phenols and terpenoids which have both beneficial and harmful effects on insect control with reference to their control needs to be documented along with economics.
2. Development of standard protocols for screening compounds for biological targets is needed (especially for fungal diseases, nematodes and bacteria).
3. Need for efficiency and efficacy of plant metabolites in critical limits on pathogens under varied climatic conditions (i.e., temperature, rainfall and relative humidity).

4. Need for more basic and applied research work on hormones/ pheromones, i.e., phytochemical changes in plants due to climate change.

HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES

INTRODUCTION

Insects are vital to the overall order of many ecosystems and have lived collectively with the human population for centuries. While insects are viewed as beneficial in several facets of society, they also endanger the human population. As agricultural pests, insect species have been the cause of food source damage and depletion, resulting in substantial economic losses. Moreover, insects serve as reservoirs for several pathogens, the causative agents of many debilitating human diseases. Dengue fever, malaria, and leishmaniasis are a few examples of human diseases transmitted by insect vectors, afflicting high morbidity and mortality on the global population, and disproportionately burdening the less-developed regions of the world due to optimal climate conditions for vector survival and reproduction, infrastructure conditions and the lack of sustained control programs.

Extracts of plant products having pesticidal components have been used to different degrees over the years which is often not in a systematic way. Progress in crop protection by chemical has been extraordinary over the last decades, not only in the invention of new and selective active ingredients but also in the assessment of the behavior of these chemicals in the environment. Scientific progress in chemistry, biology, molecular biology, and biotechnology has revolutionized the way of searching for new agrochemicals over the past decade.

Using DDT as an oldest organochlorine insecticide, the plant hormone-based phenoxy-acetic acid derivatives as different herbicides, sometimes carbamates as insecticides, different organophosphates, and thiram as a fungicide marked- at least in terms of the selective activity in pest control quantum-leap progress in pest control. We are certainly once again at the beginning of a new technological development for the protection of our traditional crops as well as in the creation of novel foodstuffs. Novel different biotechnological methods to achieve general goals are increasingly becoming available and in future these may become of greater importance, and thus the pure chemical technology of crop protection in agriculture is complementing or replacing (Bratman S. and Girman., 2003). But chemical crop protection will try to continue to play a major role in agriculture although of the emergence of new novel biotechnological solutions

Traditional method of pesticide discovery is based on the synthesis and mass screening of large numbers of compounds. Curiosity in discovering the new chemicals extended more than hundred years. This process relies exclusively on introducing as many as 20,000 compounds may have to be synthesized and examined at a cost of millions of dollars before a pesticide worthy of chemical development is identified which is both expensive and time-consuming. Further directed synthesis then strives to increase the activity of promising leads. All the pesticides in the 1940s like DDT, 2, 4-D, OPs, carbamates, thiram were introduced by these processes. The traditional approaches for discovery and screening of pesticides are Empirical synthesis/conventional screening, Analogue synthesis/ SAR optimization, Natural product models, Biochemical design and, Biorational synthesis/innovative screening.

Promising control results with most insecticides, however, have been short-lived, with the development of insecticide resistance, the culmination of factors including pesticide misuse, lack of novel compounds in the pipeline, and a dearth of diversity in the mode of action. Insecticide resistance has been reported in areas worldwide, with the most commonly used compounds such as synthetic pyrethroids, organophosphates, and chlorinated hydrocarbons, being less effective in targeting and altering the insect nervous system (WHO 2014).

Insecticide resistance, therefore, has been a contributing factor to continued persistence of agricultural pests and the resurgence of vector-borne diseases, highlighting the importance of (i) identifying and developing insecticides with alternative modes of action and (ii) alternative control approaches.

Intensive agriculture, which is associated with heavy inputs of synthetic insecticides, has serious ecological impacts, leading to loss of vital ecosystem services including insect-mediated pest suppression. In recent years, efforts have been made towards obtaining safer options to chemical insecticides for sustainable pest management. Habitat manipulation is a part of conservation biological control which aims at providing floral resources, alternative prey and shelter to predators and parasitoids to enhance and sustain natural pest suppression. The use of plant extracts as botanical insecticides is also an important provisioning ecosystem service. Integrated pest management (IPM) is an example of redesigning intensive agricultural systems. Instead of relying principally on synthetic pesticides, IPM uses non-chemical or botanical insecticide measures to suppress pest population increase and a range of curative management tactics with synthetic pesticide use as last resort (Barzman et al. 2015). The declining availability of many pesticides due to resistance and deregistration, reflecting increasing awareness of their environmental and human health consequences, has driven changes towards ecologically

based practices (Barzman et al. 2015; Borel 2017; Chagnon et al. 2015; Li et al. 2017; Sumon et al. 2018).

Ecologically based pest management tactics such as conservation biological control have been shown to reduce the use of synthetic insecticides in a variety of cropping systems whilst maintaining or increasing crop yields and efforts are being made to up scale the practice globally (Pretty et al. 2018; Wyckhuys et al. 2013; Xu et al. 2017). Despite these advantages, however, uptake of conservation biological control on a wide scale is limited (Gurr et al. 2016). In cases where uptake has been strong, the vegetation used in habitat manipulation provides multiple ecosystem services rather than suppressing pests alone (Khan et al. 2006, 2012). To date, however, there is a major gap in knowledge about the possibility of habitat manipulation plants providing botanical insecticides. This is important because synthetic insecticides present significant risks to human health. Agricultural workers and consumers are at risk of being negatively affected by insecticide products, tank mixes, drift, residues and breakdown products, especially as a consequence of poor registration, storage and misuse (Eddleston et al. 2002). In agricultural areas where there are high illiteracy rates, and poor training and equipment, the impacts are especially high (Amoabeng et al. 2017; Williamson et al. 2008).

Many plants possess secondary metabolites such as alkaloids, phenols and terpenoids that can have insecticidal activity such as toxicity, repellency, feeding deterrence against insect pests (Koul 2004). Botanical insecticides, including extracts and essential oils of these plant species, have been used to protect crops against insect herbivory for many years (Belmain et al. 2012; Isman 2000, 2008). Synthetic insecticides often have lethal and sub-lethal effects on natural enemies (Desneux et al. 2007). Biopesticides are considered relatively benign to non-target species owing to their rapid breakdown, selectivity nature and reduced risk of insecticide resistance as plant extract, particularly crude extracts have multiple modes of action other than toxicity, such as repellency (Amoabeng et al. 2013; Dubey et al. 2011; Isman 2006; Koul et al. 2008; Tembo et al. 2018). Another important benefit of botanicals is that they tend to depend on “suites” of closely related active constituents rather than a single active ingredient; this diversity may delay or mitigate the development of resistance in pest populations to most botanicals (Koul 2004). Biopesticides have been used for centuries as means of managing pests until synthetic insecticides replaced plant extracts (Isman 1997). The interest in botanical insecticides is increasing but still accounts for less than 1% of crop protectants used globally (Isman 2008, 2017). In developing countries, plant extracts are often prepared from common weed species that grow around the field and obtained freely, with labour as the only cost, resulting in cheaper pest management option when compared with synthetic insecticides (Amoabeng et al. 2014; Isman 2017).

Highthroughput screening

Highthroughput screening is a method of scientific experimentation that comprises the screening of large compound libraries for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis. High throughput is an adjective use before screening to become fastest - first and best. It represents high speed of screening throughout process and reflects what chemists can easily work upon. Use of *in-vitro* and *in vivo* assays against molecular targets for the evaluation of chemicals as lead structures in pesticide discovery (Duke J., and M. Bogenschutz, 2002). Traditional *in vivo* screening processes are time consuming, laborious, slow in process, requires larger space. *In vivo* high throughput screening permits rapidly screen large amounts of compounds for biological activity. The natural substance extracts are then automatically tested by ultra-high-throughput *in vivo* screening (UHTVS), which is performed for appropriate insecticidal, fungicidal and herbicidal efficacy. If the robots discover that a sample has the desired biological effect, it goes through further stages of isolation before being tested for its suitability as a possible pesticide (Shaon kumar Das, 2016).

Aspergillus and Rhizoctonia

Plant parasitic nematodes are harmful plant pathogens causing much more damage annually compared to insect pests, they cause projected yield loss of 12.3% ($157 billion dollars) worldwide. Out of which $40.3 million is reported from India (Singh et all., 2015). Farmers/growers identified insect pests, and other constraints as production problems but overlooked plant parasitic nematodes. Plant parasitic nematodes (PPNs) are causing serious yield loss in a wide range of plants and agriculture crops (Perry and Moens, 2011). Nematode diseases are difficult to control because of their hidden nature and hence, more often overlooked. Plant parasitic nematodes not only cause damage individually but form disease-complexes with other micro-organism and increased the crop loss. Also, the symptoms of nematode damage are not specific, resemble with the symptoms of other pathogens and abiotic stresses such as water and mineral deficiency. Future agricultural growth must come from productivity growth to address the persistent problems of poverty, food insecurity and malnutrition. Recommended measures against nematode diseases include use of clean nematode free planting material, resistant varieties, and crop rotations to suppress nematode infestation. Still the persistence of nematode has been observed and effective control needs to be obtained at faster pace in changing climatic conditions (Singh et all., 2015).

The PPNs comprise a broad range of species; so far, over 4100 species have been reported (Geng et al., 2016 them, root-knot nematodes of the genus Meloidogyne cause severe damage to most of the crucial agriculture crops and are responsible for more than 10% of yield loss worldwide, out of which $78 billion yield loss is caused by Meloidogyne incognita (Kofoid et White) Chitwood (Chen et al., 2004). Plant parasitic nematodes directly

target roots and considered to be the important agricultural pests and these encompass 15% of nematode species. (Bernard et all., 2017).

Fungal diseases in developing countries demand special attention. The general impact of fungal pathogens on human health goes beyond the ability of fungi to infect humans, since they destroy a third of all food crops annually (Fisher et al., 2012), causing economical loss and impacting global poverty. Statistics from the 2009–2010 world harvest (www.fao.org or FAOSTAT1) suggest fungi-induced losses in five of the most important crops globally (rice, wheat, maize, potatoes, and soybean). If those losses were mitigated, these crops would have been enough to feed 8.5% of the seven billion populations in 2011 (Fisher et al., 2012). The most economically devastating fungi are *Magnaporthe oryzae*, affecting rice and wheat, followed by *Botrytis cinerea*, which has a broad host range and *Puccinia spp*., affecting wheat (Dean et al., 2012). Several high-value crops produced in the tropics, such as bananas, coffee, cacao, spices, mangos, and several nuts, are currently affected by fungal infections and these crops are not produced colder climates (Drenth and Guest, 2016).

Fungal infections of invertebrate hosts may also impact agricultural crises due to ecological imbalance. For instance, bee broods are susceptible to fungal infections caused by genera of Ascosphaera and Aspergillus (Jensen et al., 2013), and the agricultural production worldwide is highly dependent on pollination mediated by bees (Aizen et al., 2009; Stein et al., 2017).

Over 19,000 fungi are known to cause diseases in crop plants worldwide. They may remain dormant but alive on both living and dead plant tissues until conditions are conducive to their proliferation. Fungal spores are readily dispersed by wind, water, soil, insects, and other invertebrates. In this way, they may infest an entire crop. Pathogenic fungi cause plant diseases such as anthracnose, leaf spot, rust, wilt, blight, coils, scab, gall, canker, damping-off, root rot, mildew, and dieback. Systemic foliar pathogens are major causes for yield and commercial crop losses and diminished crop quality. The rapid identification of fungal disease by timely recognition of their symptoms is an effective management practice and may help control and prevent their spread and progress.

Most of the crop plants are attacked by seed and soil borne diseases. Among those pathogenic fungi, Aspergillus flavus and Rhizoctonia bataticola are known to infect and causing heavy losses. The disease severity depends upon the temperature and moisture conditions. For the management of Aspergillus flavus and Rhizoctonia bataticola synthetic fungicides are known to be effective. However, the use of synthetic fungicides is limited by the emergence of resistant fungus strains and some fungicides possess considerable toxicity. Moreover, there is a growing public concern over the increased health and

environmental hazards associated with synthetic molecules. While the scientific development of new insecticides has plateaued, great strides have been made in the field of insect genomics. Emphasis is given now to develop extracts from essential oils and plants for the management of Aspergillus flavus and Rhizoctonia bataticola.

Aspergillus flavus

Taxonomic position of A. flavus

Kingdom: Fungi, Phylum: Ascomycota, Subphylum: Pezizomycotina, Class: Eurotiomycetes, Subclass: Eurotiomycetidae, Order: Eurotiales, Family: Trichocomaceae, Genus: Aspergillus, Species: flavus.

Symptomology

Aspergillus flavus colonies are commonly powdery masses of yellow-green spores on the upper surface and reddish-gold on the lower surface. In both grains and legumes, infection is minimized to small areas, and discoloration and dullness of affected areas is often seen.

Growth is rapid and colonies appear downy or powdery in texture. The conidiophores of Aspergillus flavus are rough and colorless. Phialides are both uniseriate and biseriate (Verghese., 2004). Hyphal growth usually occurs by thread-like branching and produces mycelia. Hyphae are septate and hyaline. Once established, the mycelium secretes degradative enzymes or proteins which can break down complex nutrients (food). Individual hyphae strands are not typically seen by the unaided eye; however, conidia producing thick mycelial mats are often seen. The conidiospores are asexual spores produced by Aspergillus flavus during reproduction (Horn et al., 2009 and Michelek et al., 2010).

Recently, Petromyces was identified as the sexual reproductive stage of Aspergillus flavus, where the ascospores develop within sclerotia (Amaike and Nancy., 2011). The sexual state of this heterothallic fungus arises when strains of opposite mating type are cultured together (Horn et al., 2009). Sexual reproduction occurs between sexually compatible strains belonging to different vegetative compatibility groups.

Aspergillus flavus is complex in its morphology and can be classified into two groups based on the size of sclerotia produced. Group I consists of L strains with sclerotia greater than 400 µm in diameter. Group II consists of S strains with sclerotia less than 400 µm in diameter. Both L and S strains can produce the two most common aflatoxins (B1 and B2). Unique to the S strains is the production of aflatoxin G1 and G2 which typically are not produced by Aspergillus flavus (Horn et al., 2009). The L strain is more aggressive than the S strain, but produces less aflatoxin. The L strain also has a more acidic homoeostatic point and produces less sclerotia than the S strain under more limiting conditions (Cotty., 1989).

Disease cycle

Aspergillus flavus overwinters in the soil and appears as propagules on decaying matter, either as mycelia or sclerotia. Sclerotia germinate to produce additional hyphae and asexual spores called conidia. These conidia are said to be the primary inoculum for Aspergillus flavus. The propagules in the soil, which are now conidia, are dispersed by wind and insects (such as stink bugs or lygus bugs). The conidia can land on and infect either grains or legumes. The spores enter the corn through the silks and thus infect the kernel. Conidiophores and conidia are produced in the spring from sclerotial surfaces. There is a secondary inoculum for Aspergillus flavus, which is conidia on leaf parts and leaves. Aspergillus flavus grows on leaves after damage by leaf-feeding insects. Insects are said to be a source of inoculum and promote inoculum production (Hedayati et al., 2007).

Use of natural products to control Aspergillus infection

Chemical control remains the main measure to reduce the incidence of post-harvest diseases in various foods. Antimicrobial chemicals belonging to the groups of benzimidazoles, aromatic hydrocarbons, and inhibitors of sterol biosynthesis are often used as post-harvest treatments (AL-Omair and Helaleh, 2004; Simko, 2005). Therefore, there has been increased interest in research on using natural antifungal substances, which may replace synthetic fungicides or contribute to the development of new disease control agents.

During the past 22 years, some essential oils have been shown to possess a broad spectrum of antifungal activity (Tian et al., 2011). Screening experiments with 41 aqueous and ethanolic extracts and 22 essential oils against Aspergillus section Flavi strains have shown boldo, poleo, clove, anise and thyme oils as potential antifungal candidates (Bluma et al., 2008). However, recent studies have shown that smaller compounds such as monoterpenes are most efficient when used as headspace volatiles (Avila-Sosa et al., 2011). This characteristic makes essential oils attractive as possible fumigants for the protection of stored products.

The antimicrobial efficacy of clove oil treatment of groundnuts at 50 and 100 µl/ml caused significant reductions in the Aspergillus flavus population compared to the control. In addition, A. flavus recovery after 3 days of storage was not detected. Three constituents, eugenol (89.8 per cent), caryophyllen (4.7 per cent) and vanillin (2.9 per cent) representing 98.4 per cent of the clove oil were identified. Clove oil suspensions can be used to enhance the microbial safety of groundnuts (Narumol and Jantamas, 2014).

Clove (*Syzygium aromaticum*) is widely cultivated in Indonesia, Sri Lanka, Madagascar, Tanzania and Brazil. Previous studies have shown antifungal activity of clove

oil and eugenol against yeasts and filamentous fungi, on several food-borne fungal pathogens (Lopez et al., 2005). *S. aromaticum* active ingredients of cinnamaldehyde and eugenol are noted as antifungal components against filamentous soil and seed borne fungi (Paranagama., 1991 and Jayaratne et al., 2002). In order to further clarify the spectrum of antifungal activity and its relationship to chemical composition, some general considerations must be established regarding the study of the antimicrobial activity of essential oils and the compounds isolated from them. Of the highest relevance is the definition of common parameters, such as the techniques employed, growth medium and micro-organisms tested. Standardization of both the methods of analysis of the essential oils and the assays for in vitro testing is required so that research in this area can be systematic and objective and the interpretation of results validated. The limited knowledge concerning antimicrobial activity and the mechanism of action of plant extracts has led to the addressing of such issues, although the main antifungal action of phenolic compounds, such as eugenol, appears to be exerted on the cellular membrane (Cox et al., 2001 and Carson et al., 2006).

Antifungal investigations revealed that garlic extract was effective against oilseed-borne toxigenic Aspergillus and Penicillium species (Ikeura et al., 2011 and Tagoe et al., 2011). In a study by Muhsin et al., (2001) growth of 18 different fungal species was effectively inhibited by crude garlic bulb extract. Antifungal activity of garlic juice could be attributed to its phytochemical properties (Obagwu., 2003). Garlic allicin decomposes into several effective compounds, such as diallylsulphide, diallyldisulphide, diallyltrisulphide, allyl methyl trisulphide, dithiins and E,Z-ajoene, that serve as antimicrobial agents (Jabar and Mossawi., 2007). Inhibitory effects of garlic juice against Aspergillus and Penicillium fungi suggest the possible use of garlic in controlling food-spoiling fungi. Meanwhile, the use of water-based juice provides an alternative to chemical solvents, which can be toxic at certain concentrations. Garlic juice was capable of inhibiting fungal growth, and it can be used as a source of antifungal compounds to prevent fungal infections of stored groundnuts (Tagoe et al., 2011).

Vanilla is widely used in flavoring materials worldwide and is the second most expensive spice in the world next to saffron (Lubinsky et al., 2008). Despite its broad utilization it had not been seriously researched for any bioactivity. However, it has been reported by some few researchers that vanilla might possess antimicrobial activity (Beuchat and Golden., 1989). Jay and Rivers (1984) found that vanilla was very active in suppressing moulds and non-lactic Gram positive bacteria. Lopez-Malo et al., (1995) investigated with different fruit based agar media containing mango, papaya, pineapple, apple and banana with 2000 µg/ml vanillin and incubated each with *A. flavus*, *A. niger*, *A. ochraceus*, or *A. parasiticus*. Vanillin concentration at 1500 µg/ml significantly inhibited all the strains of Aspergillus in all media. However, vanillin had less effectiveness in banana

and mango agars. Vanillin has also been reported to possess anti-clastogenic, anti-mutagenic and anti-tumour properties and, therefore, it can be considered as a nutraceutical molecule (Sinigaglia et al., 2004 and Shyamala et al., 2007). The antimicrobial property of vanillin is the effect of a phenolic compound which makes vanillin effective in inhibiting bacteria, yeasts and moulds. It is structurally similar to eugenol (2-methoxy-4-(2-propenyl) phenol) from clove and is known to be antimycotic (Beuchat and Golden., 1989) and bacteriostatic (Fitzgerald et al., 2004). At low concentrations, phenols affect enzyme activity, especially those enzymes associated with energy production, while at greater concentrations they cause proteins to denature (Prindle and Wright., 1977).

In other work on Aspergillus infection of groundnut, Mondali et al., (2009) reported the efficacy of aqueous and alcoholic extracts of neem leaf, garlic, ginger and onion against seed-borne A. flavus, which showed that treatments were effective in inhibiting the pathogen. Srichana et al., (2009) screened the efficacy of betel leaf extract on the growth of A. flavus, and it was shown that the extract at 10,000 ppm concentration was highly significant in suppressing the tested pathogen. This, however, is an extremely high concentration and the commercial viability of this material must be questioned.

Chatterjee (1990) showed that cassia, clove star-anise, geranium, and basil prevented the infection of maize seed in vitro by *Aspergillus flavus*, *A. glaucus*, *A. niger*, and *A. sydowi*.

Bansal and Sobti (1990) reported that neem extract was most effective plant extract in reducing the Aspergillus flavus incidence on groundnut seeds by application of two per cent aqueous solution of neem. The incidence of Aspergillus flavus on groundnut seeds was 2.66 per cent as compared to control with 9 per cent.

Srivatsava et al. (1997) studied the antifungal activity of neem (*Azadirachta indica* L.) and karanj (*Pongamia pinnata* L.) seeds and neem leaves against A. flavus and A. niger. Among different treatments, neem seeds treated with neem seed oil (NSO) was found best in inhibiting the mycoflora of neem seeds. The bioefficacy of neem seed kernel powder (NSKP), karanj seed kernel powder (KSKP) and neem leaf powder (NLP) were more effective when pelleted on wet seeds as compared to the dry seeds. The NSO and NLP inhibited the growth of Aspergillus flavus.

Montes-Belmont and Carvajal (1998) evaluated the fungicide properties of *Cinnamomum zeylanicum, Mentha piperita, Ocimum basilicum, Origanum vulgare, Teloxysam brosioides, Syzygium aromaticum*, and *Thymus vulgaris*. These plant essential oils controlled A. flavus growth on artificially infected maize seeds germinated in the laboratory. Corn seedlings treated with these essential oils did not exhibit phytotoxicity.

The essential oils of 12 medicinal plants viz. anise (*Pimpinella anisum*), caraway (*Carum carvi*), fennel (*Foeniculum vulgare*), thyme (*Thymus vulgaris*), spearmint (*Mentha spicata*), basil (*Ocimum basilicum*), chamomile (*Chamomilla recutita*), marigold (*Calendula officinalis*), hazanbul (*Achillea millefolium*), qyssum (*A. fragrantissima*), ghafath (*Agrimonia eupatoria*) and cinnamon (*Cinnamomum zeylanicum*) were tested for inhibitory activity against *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme*. The oils of thyme and cinnamon (<=500 ppm), marigold (<=2000 ppm), spearmint, basil and qyssum (3000 ppm) completely inhibited all the tested fungi. Caraway was inhibitory at 2000 ppm against *A. flavus* and *A. parasiticus*, and at 3000 ppm against *A. ochraceus* and *F. moniliforme*. *A. flavus*, *A. ochraceus*, *A. parasiticus* and *F. moniliforme* were completely inhibited by anise <=500 ppm. However, chamomile and hazanbul at all concentrations were partially effective against the test toxigenic fungi (Soliman and Badea., 2002).

Paranagama et al. (2003) developed a natural fungicide against aflatoxigenic fungi, using the essential oil of lemongrass. They isolated Aspergillus flavus from stored rice and identified as aflatoxigenic grain. Lemongrass was tested against Aspergillus flavus and the test oil was fungi static and fungicidal against the test pathogen at 0.6 and 1.0 mg/ml, respectively. The results obtained from the thin layer and gas chromatography indicated citral A & B as fungicidal constituents in lemon grass oil. During the fumigant toxicity assay of lemongrass oil, the sporulation and the mycelia growth of the test pathogen were in habited at the concentrations of 2.80 and 3.46 mg/ml respectively.

Five essential oils extracted from *Cymbopogon citratus, Monodora myristica, Ocimum gratissimum, Thymus vulgaris* and *Zingiber officinale* were investigated for their antifungal effect against food spoilage and mycotoxin producing fungi, *Fusarium moniliforme, Aspergillus flavus* and *A. fumigatus*. The essential oils from *O. gratissimum, T. vulgaris* and *C. citratus* were the most effective at 800, 1000 and 1200 ppm, respectively (Nguefack et al., 2004).

The oils of rosewood, bay, sassafras, and cinnamon inhibited the growth of *Aspergillus sp., Fusarium sp*., and *Penicillium sp*. in vitro (Simic et al., 2004). Cinnamon essential oil was the most effective at controlling the pathogen growth. GC-MS characterization showed that the essential oils had different composition. The antifungal activity of the oils was based on the interactions among the different compounds, rather than a few compounds providing the antifungal properties to all oils.

Reddy et al. (2004) evaluated different botanicals/ plant extracts for the inhibition of fungal growth of Aspergillus flavus. Among the different plant extracts *Allium sativum* (bulb), *Azadirachta indica* (leaf), *Eucalyptus terticornis* (leaf), *Pongamia pinnata* (kernel)

extracts and garlic bulb extract (5%) completely inhibited the fungal growth of Aspergillus flavus. Pongamia kernel extract gave control of 57 per cent of Aspergillus flavus at 20 per cent concentration. Eucalyptus (40%) and neem extract (28%) were less effective in inhibiting the growth of Aspergillus flavus.

Ajith Kumar et al. (2005) reported 100 per cent inhibition of Aspergillus flavus by use of neem seed kernel extract, nimbicidin and pongamia oil at 5 per cent concentration under in vitro in chilli.

Extract from *Garcinia pedunculata*, with a high content of phenolic acids, inhibited the growth and aflatoxin production in *A. parasiticus* and *A. flavus*. The extract from Garcinia, have a rich source of phenolic acids that inhibited more aflatoxin production than growth of Aspegillus section Flavi (Joseph et al., 2005).

Pawar and Thaker (2006) screened seventy-five essential oils for their antifungal properties against *Aspergillus niger*. Only five oils controlled pathogen growth. Essential oils extracted from *Cinnamomum zeylanicum* (bark and leaf), *Cinnamomum cassia*, *Syzygium aromaticum*, and *Cymbopogon citratus* had good antifungal activity, while cinnamon bark oil had the best control rate.

Satish et al. (2007) studied the aqueous extract of 52 plants from different families for their antifungal potential against eight important species of Aspergillus. Aqueous extract of *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopisjuliflora, Punica granatum* and *Sygigium cumini* have very good antifungal activity against one or the other *Aspergillus species* tested. Among the solvent extracts tested, methanol gave more effective than ethanol, chloroform, benzene and petroleum ether.

The essential oils from 12 medicinal plants were tested to inhibit the Aspergillus parasiticus. The fungus was cultured in presence of various oils in 6-well microplates using a micro bioassay technique. The mycelial growth was estimated. Among essential oils tested, *Thymus vulgari* and *Citrus aurantifolia* were found to inhibit both *A. parasiticus*. The essential oils from *Mentha spicata* L., *Foeniculum miller, Azadirachta indica* A. Juss, *Conium maculatum* and *Artemisia dracunculus* were also inhibited fungal growth but not as *Thymus vulgari* and *Citrus aurantifolia*. These results indicate that the essential oils of some medicinal plants may be considered as potential candidates to protect foods and feeds from toxigenic fungus growth (Razzaghi-Abyaneh et al., 2009).

Adjou et al. (2012) studied antifungal activity of *Ocimum canum* essential oil against toxicogenic fungi. The essential oil was found to be strongly fungicidal and inhibitory to aflatoxin production. Through GC/MS analysis, an amount of 30 components were identified, representing almost 95.2% of the oil. Essential oil of *O. canum* was characterized by major components such as terpinene-4-ol (41.18 %), linalol (14.7 %) and terpene (6.9 %). This plant offers novel approach to the management of storage fungi.

Passone et al. (2012) reported on the antifungal activity of five plant essential oils: from boldo *Peumus boldos* Molina, *Poleo Lippia* turbinate var. integrifolia, (Griseb.), clove *S. aromaticum* L., anise *Pimpinella anisum* and thyme *Thymus vulgaris*. These were tested against aflatoxigenic strains of *A. flavus* and *A. parasiticus* on groundnut-based media, conditioned at different water activities of 0.98, 0.95, 0.93. The effects of EOs were assessed, when the oils were applied to groundnut meal extract agar, by recording the lag phase, growth rate, and aflatoxin B1 accumulation of the tested pathogens. The results showed that lowest concentration (500 ppm) had no effect on the pathogens, but higher concentrations (2500 µl-1 for boldo and poleo; 1500 µl-1 for clove) completely inhibited the growth of *Aspergillus spp*. irrespective of the medium.

Sudha et al. (2013) evaluated the effect of different plant extracts viz., neem (*Azadirachta indica*) seed kernel extract (NSKE), Pongamia (*Pongamia pinnata*) oil and nimbicidin and rakshak at 1.0, 2.5 and 5.0 per cent concentration against Aspergillus flavus in chilli using the poisoned food technique. Cent % inhibition (100 %) of Aspergillus flavus was reported with NSKE, nimbicidin and pongamia oil and least inhibition of growth in rakshak (93.3 %) at 5 per cent.

Srilakshmi et al. (2013) investigated the bioactivity of secondary metabolites or small molecules produced by *Trichoderma spp*. and their efficacy against aflatoxin contamination in groundnut. The results indicated that 48 strains of Trichoderma were highly significant in suppressing an *A. flavus* isolate (AF11-4) and subsequently reduced aflatoxin production in groundnut. It is also feasible to apply BCAs in combination, sometimes including bacterial and fungal antagonists.

The antimicrobial activity of essential oils depends on the combination and proportions of different compounds found in their composition. Monoterpenes are the main constituents of essential oils, and many of them have been reported for their antifungal, anti-aflatoxin and antioxidant activity. Studies showed that essential oils possess antifungal activity being able to inhibit the aflatoxin production (Kedia et. al., 2014). The antifungal activity of essential oils was studied and reported using different methods as there are no standard protocols to test the effect of this naturally compounds on fungi.

Rhizoctonia bataticola

Taxonomic position of Rhizoctonia bataticola

Kingdom: Fungi, Phylum: Basidiomycota, Subphylum: Aquaricomycotina, Class: Basidiomycetes, Subclass: Aquaricomycetidae, Order: Polyporales, Family: Corticiaceae, Genus: Rhizoctonia, Species: bataticola.

Symptomology

Grover and Sakhuja (1981) reported leaf blight phase of mung bean with the information that disease usually makes its appearance when the crop is 4-6 weeks old and the older leaves are first affected. Initially small, circular to irregular brown to reddish brown lesions appears on or near the margins which enlarge and coalesce. Under hot and humid conditions the entire plant may be blighted. The dried lesions are yellowish brown and become papery in texture. Severely affected leaves fall-off prematurely.

Nene et al. (1978) observed continuous black discoloration of pith and xylem vessels of roots and basal shoots as one of the main characteristic symptoms of dry root rot of chickpea. Sanecha and Srivastava (1982) reported that in affected young seedlings of cowpea, discoloration and rotting occurred from young root tip and proceed backwards. The cotyledonary leaves are completely blighted and necrotic. Stem decay occurs in advance stage.

Baldev et al. (1988) reported symptoms of dry root rot of chickpea which consisted of straw colored leaves and stems. Tap root of infected plants was observed to be dry and devoid of lateral and finer roots. Roots were turned dark and showed signs of rotting. The dead root was observed brittle towards the tip and showed shredding of bark. Haware (1990) reported that dry root rot of chickpea appears around flowering and podding time in the form of scattered dried plants but seedlings are also infected. The symptoms induced were drooping of petioles and leaflets which were confined to top of the plants. Shredding of bark in form of flakes was observed. Singh et al. (1990) observed that Rhizoctonia bataticola inoculated roots of chickpea, upon microscopic examination, showed disintegration of cortical tissue and plugging of xylem vessels with mycelial and sclerotial bodies of fungus.

Rangaswamy (1996) reported field symptoms which include yellowing of plants with drooping of leaves. Due to decay of roots, plants can be easily uprooted. Root portion appears brownish from outside. The stem and root below the region shows rotting with frequently pinkish white mycelial growth. Dried plants scattered throughout the field are indicative of root rot incidence.

Singh (1999) reported that dry root rot of chickpea occurred from flowering to podding stage. Infected plants were suddenly dried in the field. The tap roots turned dark brittle and devoid of lateral roots. Khalid and Ilyas (2000) considered the presence of root lesions and sclerotium plugging in xylem vessels of roots and collar region as symptoms of dry root rot of chickpea for screening of germplasm against this disease. Singh and Agarwal (2002) observed the withering and drying of chickpea plants in the field due to the infestation of Rhizoctonia bataticola.

Disease cycle

Rhizoctonia bataticola can survive for many years by producing small (1 to 3-mm diameter), irregular-shaped, brown to black structures (called sclerotia) in soil and on plant tissue. Rhizoctonia bataticola have evolved the ability to produce sclerotia with a thick outer layer that allows them to float and survive in water. Rhizoctonia bataticola also survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity. Sclerotia and/or mycelium present in soil and/or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of food and fiber crops. The fungus is attracted to the plant by chemical stimulants released by actively growing plant cells and/or decomposing plant residues. As the attraction process proceeds, the fungal hypha will come in contact with the plant and become attached to its external surface. After attachment, the fungus continues to grow on the external surface of the plant and will causes disease by producing a specialized infection structure (either an appresorium or infection cushion) that penetrates the plant cell and releases nutrients for continued fungal growth and development. The infection process is promoted by the production of many different extracellular enzymes that degrade various components of plant cell walls (e.g. cellulose, cutin and pectin). As the fungus kills the plant cells, the hyphae continue to grow and colonize dead tissue, often forming sclerotia. New inoculum is produced on or in host tissue and a new cycle is repeated when new substrates become available.

Use of natural products to control Rhizoctonia infection

Singh et al. (1980) studied the effect of aqueous extract and oil of neem (*Azadirachta indica*) on soil borne pathogen Rhizoctonia bataticola, causing dry root rot in chickpea (*Cicer arietinum*). Growth of pathogen, in liquid media, was inhibited by extracts of leaf, trunk, bark fruit pulp and oil. Out of them, neem oil showed maximum inhibitory effect.

Singh et al. (1980) and Bhaskar et al., (2005) reported that neem (*Azadirachta indies*) leaf extracts effective in inhibiting the mycelial growth of Rhizoctonia bataticola.

Kishore et al. (1982) screened 31 plant species to evaluate their fungi toxicity against Rhizoctonia bataticola. The leaves of Allamanda cathartics and *Artabotrys hexapetalus* showed absolute toxicity against Rhizoctonia bataticola. Sindhan and Jaglan., (1988). Reported that effectiveness of neem as well as *Allium cepa* and *A. sativam* against Rhizoctonia bataticola.

Mishra and Tiwari (1992) tested the leaf extract of *Polyanthia longifolia* against Rhizoctonia bataticola and reported significant reduction in the mycelial growth of Rhizoctonia bataticola in *Polyanthia longitfolia*.

Ansari (1995) reported antifungal activity of ajowain (*Trachispermum ammi*), lemon grass (*Cymbopogon citratus*), Tulsi (*Ocimum sp.*), mentha (*Mentha sp.*), *Rauwolfia sp*., mehandi (*Lawsonia inermins*), and samhalu (*Vertex trifolia*). All the medicinal plants significantly reduced the growth of Rhizoctonia bataticola.

Shivpuri et al. (1997) worked out the toxicity of extracts of ten plant species (*Allium sativam, Allium cepa, Azadirachta indica, Calotropis procera, Datura strumoniun, Ocimum sanctum, Polyanthia longifolia, Tagetes erecta, Vinca rosea* and *Withania somnifera* under laboratory conditions and found fungitoxic properties of these botanicals against Rhizoctonia bataticola.

Sindhan et al. (1999) tested nine plant extracts against mycelial growth of *Macrophomina phaseolina* and *Rhizoctonia solani* in vitro at 5, 10 and 20 per cent concentration. All plant extracts were found inhibitory to *Rhizoctonia solani* and *M. phaseolina* at all concentrations. Both fungi were found more sensitive to extracts of onion, ginger, neem, garlic followed by mint, eucalyptus and tulsi.

Kaushal et al. (2003) tested 24 botanicals belonging to the family Compositae for their fungitoxicity against Rhizoctonia bataticola. The highest percent inhibition of mycelial growth was observed at 1000 µg/ml followed by 200 µg/ml at 48 and 60 hrs respectively.

Sharma et al. (2005) have demonstrated the efficacy of plant extract of *Eucalyptus globulus* against Rhizoctonia bataticola on gram. There was 85 per cent reduction in sclerotial formation when ten per cent plant extract was used. Leaf extract of neem (*Azadirachta indica*) was found effective in inhibiting the mycelial growth of Rhizoctonia bataticola in chickpea (Kshirsagar et al., 2004; Sharma et al., 2005; Shahraj et al., 2007).

Tandel et al. (2010) tried phyto-extracts of eleven plant species against Rhizoctonia bataticola of gram and revealed that the onion bulb extract produced maximum inhibition (98.14 %) followed by extract of acacia, ginger, neem, garlic and karanj.

Kumar et. al. (2011) tested neem leaf extract (20 %) and found effective in inhibiting (55.6 %) mycelium growth of Rhizoctonia bataticola, causing root rot of jatropa.

Evaluated the antifungal activity of essential oil of Eucalyptus (*Camaldulensis dehnh*) against five Fusarium spp. commonly associated with maize. The essential oil produced complete mycelial growth inhibition in all the test pathogens at a concentration of 7-8 l/ml after five days of incubation. The minimum inhibitory concentration and minimum fungicidal concentration of the essential oil on the test fungi were in the range of 7-8 l/ml and 8-10 l/ml, respectively. These findings confirm the fungicidal properties of *E. camaldulensis* essential oils and their potential use in the management of economically important *Fusarium spp*. and as possible alternatives to synthetic fungicides (Martin et al., 2017).

Keeping the above in view, the project, "**HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES"** was taken up by Department of Molecular Biology and Agricultural Biotechnology, University of Agricultural Sciences, Raichur with Rashtriya Krishi Vikas Yojana funding. The project was implemented during 2016-17. The details of the project are as under:

1.	**Title of Project**	**:**	"**HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/ NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES"**
2.	**Nodal officer and Principal Investigator**	**:**	**Dr. B. Kisan,** Asst. Professor and Head, Department of Molecular Biology and Agricultural Biotechnology, College of Agriculture, University of Agricultural Sciences, Raichur
3.	**Implementing Institution (S) and other collaborating Institution (s)**	**:**	Department of Molecular Biology and Agricultural Biotechnology, University of Agricultural Sciences, Raichur
4.	**Date of commencement of Project**	**:**	2016-17
5.	**Approved date of completion**	**:**	2016-17
6.	**Actual date of completion**	**:**	2016-17

7.	**Project cost**	**:**	Rs. 30 lakhs

The objectives of the project are as follows:

1. Obtaining chemical scaffolds/ molecules for screening.
2. Developing highthroughput screening platform.
3. Testing the molecules for their effectiveness.
4. Evaluation of efficacy on major insect/ pests.

HYPOTHESIS

The context of the evaluation arises from the following facts:

1. Insects are vital to the overall order of many ecosystems and have lived collectively with the human population for centuries. While insects are viewed as beneficial in several facets of society, they also endanger the human population. As agricultural pests, insect species have been the cause of food source damage and depletion, resulting in substantial economic losses.
2. Progress in crop protection by chemical has been extraordinary over the last decades, not only in the invention of new and selective active ingredients but also in the assessment of the behavior of these chemicals in the environment. Scientific progress in chemistry, biology, molecular biology, and biotechnology has revolutionized the way of searching for new agrochemicals over the past decade.
3. Promising control results with most insecticides, however, have been short-lived, with the development of insecticide resistance, the culmination of factors including pesticide misuse, lack of novel compounds in the pipeline, and a dearth of diversity in the mode of action. Insecticide resistance has been reported in areas worldwide, with the most commonly used compounds such as synthetic pyrethroids, organophosphates, and chlorinated hydrocarbons, being less effective in targeting and altering the insect nervous system.
4. Many plants possess secondary metabolites such as alkaloids, phenols and terpenoids that can have insecticidal activity such as toxicity, repellency, feeding deterrence against insect pests. Botanical insecticides, including extracts and essential oils of these plant species, have been used to protect crops against insect herbivory for many years.
5. Highthroughput screening is a method of scientific experimentation that comprises the screening of large compound libraries for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis. High throughput is an adjective use before screening to become fastest - first and best. It represents high speed of screening throughout process and reflects what chemists can easily work upon. Use of *in-vitro* and *in vivo* assays against molecular targets for the evaluation of chemicals as lead structures in pesticide discovery.

6. Most of the crop plants are attacked by seed and soil borne diseases. Among those pathogenic fungi, Aspergillus flavus and Rhizoctonia bataticola are known to infect and causing heavy losses. For the management of Aspergillus flavus and Rhizoctonia bataticola synthetic fungicides are known to be effective. However, the use of synthetic fungicides is limited by the emergence of resistant fungus strains and some fungicides possess considerable toxicity. Moreover, there is a growing public concern over the increased health and environmental hazards associated with synthetic molecules. While the scientific development of new insecticides has plateaued, great strides have been made in the field of insect genomics. Emphasis is given now to develop extracts from essential oils and plants for the management of Aspergillus flavus and Rhizoctonia bataticola.

OBJECTIVES AND ISSUES FOR EVALUATION

The scope of evaluation is to study the impact of scheme, "**HIGHTHROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/ NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES"** implemented by University of Agricultural Sciences, Raichur during the period 2016-17.

1. Stake Holders

a) University of Agricultural Sciences, Raichur – Sponsorer
b) Rashtriya Krishi Vikas Yojane – as Monitoring Authority
c) Institution of Agriculture Technologists – as Consultant
d) Farmers / beneficiaries as target group of evaluation

2. Purpose of Evaluation

Evaluation Framework

The focus of Evaluation is:

i. To examine whether the screening procedure based on 96 well plate and utility of imaging and other methodologies adopted was able to expand the screening procedure faster, cheaper and in timely fashion.
ii. To evaluate plant extracts, essential oils and synthetic chemical compounds to screen for their activity against the nematodes and fungal pathogen inhibition.
iii. To evaluate the efficacy of molecules selected on control of diseases

LOG FRAME/THEORY OF CHANGE/PROGRAM THEORY

The intention of the scheme is to develop a screening procedure using sophisticated systems and assays that can evaluate plant extracts, essential oils and synthetic chemicals for their activity against nematodes and fungal pathogens and evaluating the efficacy of the molecules for control of diseases in the field. The underlying logic is;

a. Screening of large compound libraries for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis becomes fastest - first and best.
b. It represents high speed of screening throughput process and reflects what chemists can easily work upon.
c. Emphasis on use of plant extracts and essential oils in place of synthetic chemical compounds to screen for their activity against the nematodes and fungal pathogen inhibition will pave the way for development of pest management systems that minimize health and environmental hazards.

EVALUATION DESIGN

Evaluation design has a rationale of requirement of field level data (primary) that is required to study evaluation objective with respect to beneficiary farmers on one part and the projects taken up for study per se on the other part. The evaluation requires analysis of administration obligations under the two heads and hence a secondary data analysis becomes important and accordingly formats were designed to procure secondary data. The third obligation under evaluation is opinion of stake holders with respect to improvement of the schemes, which require group discussions and exchange of views both in the form of a format, as well as group discussions with the stake holders. The entire evaluation process required a central administration of all activities.

A core team of experts at the Institution level considered three methods to bring a meaningful evaluation of the subject, keeping in mind the scope, evaluation questions and sub-questions duly keeping its focus on the purpose of evaluation. The three methods are:

a. Accessing and analysis of secondary data from the implementing department.
b. Interaction with Principal Investigator and his team.
c. Actual visit to the project site to study and obtain necessary information to elicit answers to the evaluation questions.

DATA COLLECTION AND ANALYSIS

The research work was carried out during 2017-18 at the Department of Molecular Biology and Agricultural Biotechnology, College of Agriculture and Main Agricultural Research Station, University of Agricultural Sciences Raichur, Karnataka (India).

The identified pure culture of pathogenic fungi Aspergillus flavus and Rhizoctonia bataticola were collected from Department of Plant Pathology, College of Agriculture, UAS, Raichur for the study. The Aspergillus flavus and Rhizoctonia bataticola cultures were subcultured on PDA slants and kept at 28 ± 2 ºC for 7 days. Those slants were preserved in refrigerator at 4 ºC and maintained by sub-culturing once in a month in order to avoid a decline in strain viability. Such cultures were used throughout the study.

To prepare the culture media, 200 gm of peeled potatoes were cut into small pieces and boiled in distilled water. The extract was collected by filtering though muslin cloth. Dextrose 20 gm and agar 20 gm each were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water and sterilized and preserved for further use.

Potato dextrose broth was prepared similarly but without adding agar and volume was made upto 1000 ml with distilled water, sterilized and preserved for further use.

Essential Oils

Different essential oils were obtained from different companies as mentioned below.

Orange oil	Peppermint oil	Citronell oil	Methyl eugenol
Eugenol	Aijawain seed oil	Cumin seed oil	Rose oil
Lavender oil	Clove oil	Cinnamon oil	Eucalyptus oil

Dilution of Essential Oils

Each essential oil was diluted at different concentrations such as 0.05, 0.1, 0.25, 0.5, 1 and 2 per cent in 10 ml of Potato Dextrose Broth and 10 µl of Tween-20 (Sigma-Aldrich) to dissolve essential oil in water.

Plant extracts

The following plant extracts were collected from Jain University Bengaluru for the study:

Azadirachta indica leaf	*Centella aciatica*	*Ocimum gratissimum*
Ocimum tenuiflorum	*Aeonium arboreum*	*Styrax spp.*
Laguncularia recemosa	*Euphrasia offcinalis*	*Tecoma stans*
Duranta erecta	*Urtica dioica*	*Morinda citrifolia*
Justicia adhatoda	*Vitex nigundo*	*Vinca major*
Lawsonia inermis	*Gloriosa superba*	*Lonicera japonica*
Aloe vera	*Baccharis trimera*	*Cordia verbenacea*

Euphorbia tirucalli	*Juglans regia*	*Leonotis nepetaefolia*

Synthetic chemicals

A total of 64 synthetic chemicals in 4 batches were obtained from Kuvempu University, Shivamogga for screening.

Screening of essential oils against A. flavus and R. bataticola by using Microtiter plate

A simple technique which enables the monitoring of fungal growth with the aid of a microplate reader was followed according to Willem et al. (1989). Sterile 96-well plate was labelled (Fig.2), Culture of fungi for the microplate reader assay was done in sterile flat bottom microtiter plates at 28 ± 2 °C. 300µl of distilled water was taken in 1st column, Controls (290 µl of PDB + 10 µl of *A. flavus/R. bataticola* culture) were maintained without essential oils in a 2nd column and Test samples with different concentrations of essential oils (290 µl of PDB with essential oils + 10 µl of *A. flavus/R. bataticola* culture) were maintained in next columns and performed in triplicates. All the microtiter plates were sealed with para film and incubated at 28 ± 2 ºC. Microplate readings were taken in Micro plate reader (Bio-Rad) immediately after inoculation and every 24 hr for 7 days at 595 nm.

In vitro evaluation of essential oils against *A. flavus and R. bataticola*

The experiment was carried out in completely randomized design. The efficacy of each essential oil was evaluated against *A. flavus and R. bataticola* under in vitro conditions on the Potato Dextrose Agar media using poison food technique (Nene and Thapliyal, 1993). The required quantities of individual essential oils were added separately into molten and cooled Potato Dextrose Agar to get the desired concentration (Control, 0.05 %, 0.1 %, 0.25 %, 0.5 %, 1 % and 2 %) of the essential oils. Later 2 ml of such poisoned medium was poured into sterile Petri plates. 10 µl of seven day old liquid culture of the *A. flavus/R. bataticola* was spotted on to the media at the center of the Petri plates. Control was maintained without adding any essential oil to the medium and each treatment was replicated thrice. Then such plates were incubated at 28 ± 2 ºC temperature for seven days and radial colony growth (cm) was measured. The efficacy of an essential oil was expressed as per cent inhibition of mycelial growth over control that was calculated by using Vincent's formula (1947). The per cent values were converted into angular transformations and the data were analyzed statistically by Factorial Completely Randomized Design analysis.

Preparation of Resazurin solution

0.02 per cent of Resazurin solution was prepared by dissolving 2 mg in 10 ml of sterile distilled water. The solution was vortexed and dissolved to make homogenous solution.

Preparation of the plates

Plates were prepared under aseptic conditions. The plates were prepared in triplicate. A sterile 96-well plate was labelled. 180 µl of diluted essential oil samples were loaded according to the label and 20 µl of *A. flavus/R. bataticola* culture was added into all the wells, controls were maintained without essential oils. The plates were sealed with para film and incubated at 28 ± 2 ºC for 5 days. After 5 days of incubation 20 µl of 0.02 per cent Resazurin solution was added into all wells and incubated for 30 min. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value (Bonnier et al., 2015).

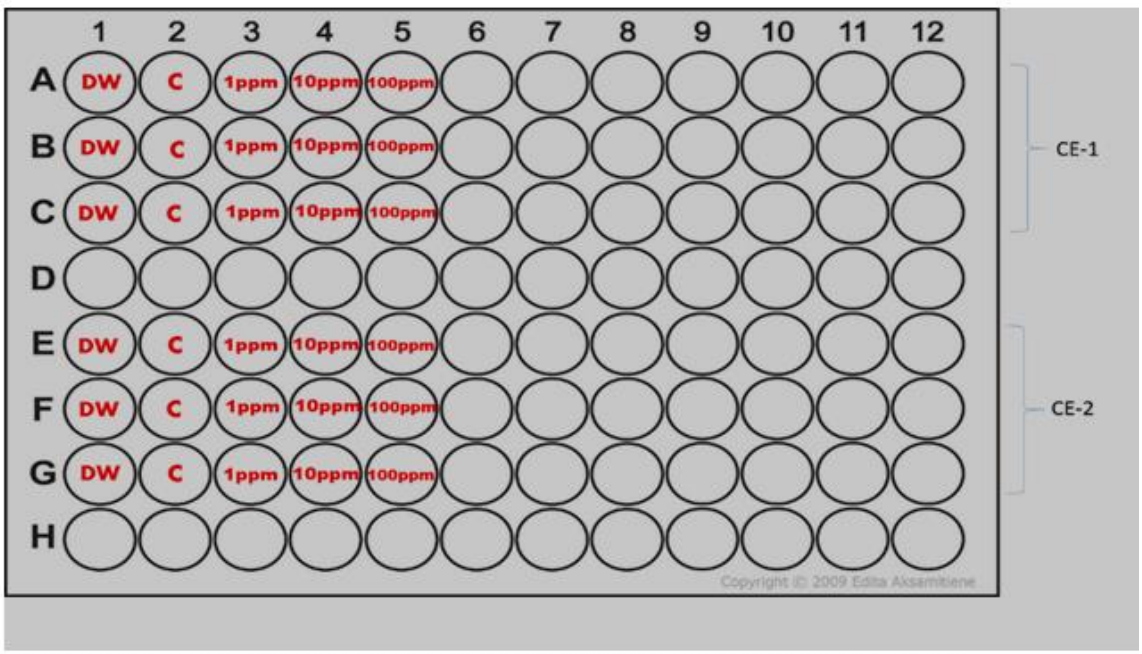


Fig: Figure showing 96-well format for Resazurin assay for synthetic chemicals

DW- Distilled water, C- Control

Pyocyanin assay

Pyocyanin was extracted from 5 days grown *A. flavus/R. bataticola* culture supernatant (Essar et al., 1990). Potato Dextrose Broth media was prepared and *A. flavus/R. bataticola* culture was inoculated. Grown the *A. flavus/R. bataticola* cultures in treated (control, 0.5 %, 1 % and 2 % with 5 effective essential oils). Incubated at 28 ± 2 ºC in shaker for 5 days. After the 5 days of growth, the supernatant was collected and 5 ml of supernatant from each flask was mixed with 3 ml of chloroform and mixed vigorously. The chloroform layer was mixed with 1ml of 0.2 M HCl. After centrifugation (10 min, 28 °C, 8,000 rpm), the OD of the HCl layer was measured at 520 nm against 0.2 M HCl using an UV/Vis spectrophotometer (Eppendorf).

Estimation of peroxidase (POX) activity

POX catalyzes the dehydrogenation of a large number of organic compounds as phenols and aromatic amines. It was determined following the dehydrogenation of guaiacol as a substrate according to Malik and Singh (1980).

Nematicidal activity:

For nematicidal activity the methodology used was moving versus non-moving nematodes were counted with Model nematode *Caenorhabditis elegans* for the preliminary screening and for the final testing root knot causing nematode *Meloidogyne incognetta* was utilised in the study and also acridine orange was also stained to confirm live and dead nematodes.

Caenorhabditis elegans is a nematode worm and is significantly anatomically simpler than a human. However, it does share many similarities at the molecular level making it a good candidate for a model organism.

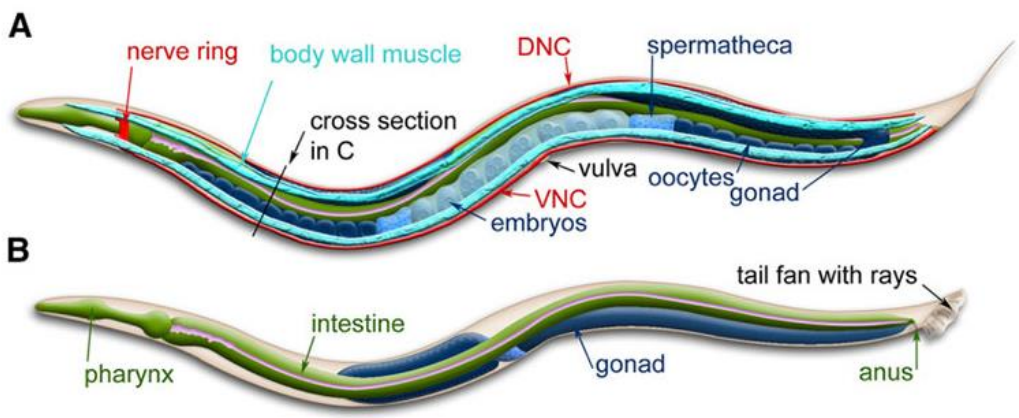


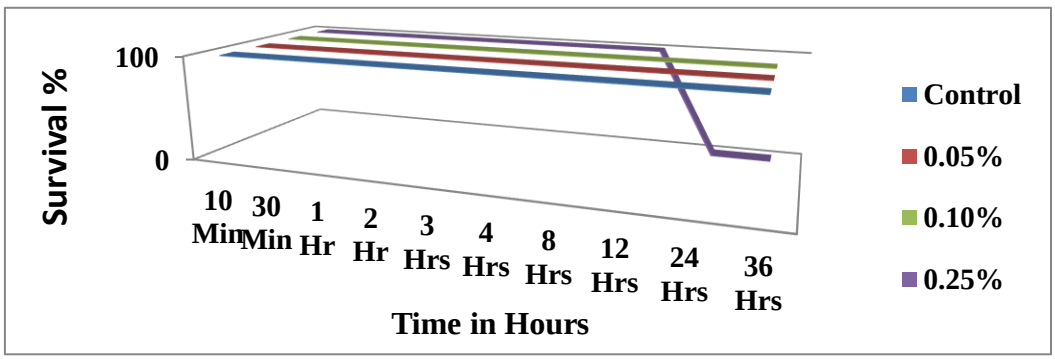
Figure: *Caenorhabditis elegans* anatomy. Major anatomical features of a hermaphrodite (A) and male (B) viewed laterally.

As with most of the other model organisms considered, *Caenorhabditis elegans* is easy to look after, feed and maintain throughout their 2-week lifespan. *Caenorhabditis elegans* has few discernible external features, and as such, initially appears a poor subject for study. However, it does have many advantages: its transparency makes it possible to observe the fate of individual cells using simple microscopy, or more advanced techniques such as SPIM or Light Sheet Microscopy. *Caenorhabditis elegans* grown in large numbers, can be easily screened for effects of novel drugs on complex processes involved in human disease. *Caenorhabditis elegans* is particularly useful for the study of ageing processes because the organism passes through several distinct phases of life which can be observed physiologically and genetically. Moreover, *Caenorhabditis elegans* is also used to study

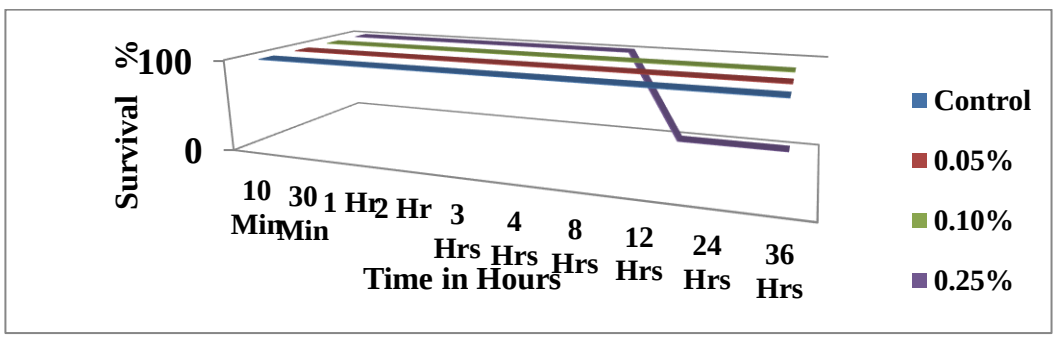
neural development; it is well suited for this application due to the availability of a comprehensive connectivity map and only 302 neurons and ~7000 synapses.

FINDINGS AND DISCUSSION

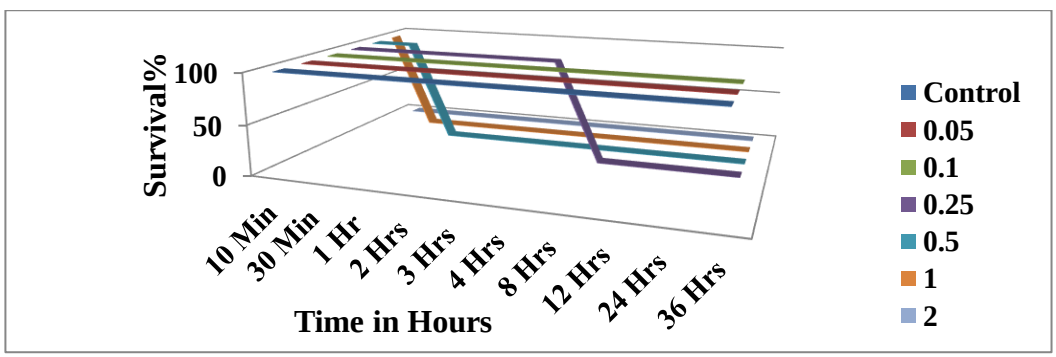
Among the essential oils (EOs) tested, OL-2 and OL-12 combination of EOs for concentrations 0.25, 0.5, 1 and 2 per cent, cent percent mortality observed at time intervals 10 min onwards. The essential oils independently brought about 100% mortality within 12 hours of incubation.



OL-2



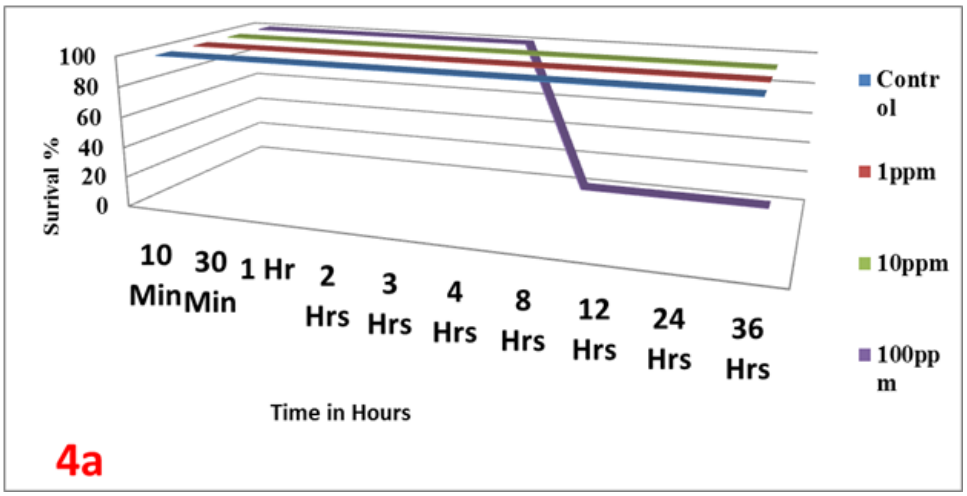
OL-12

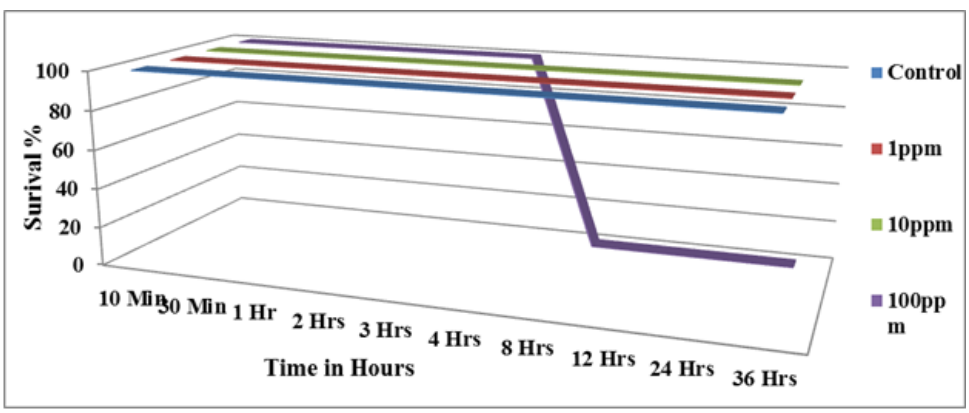


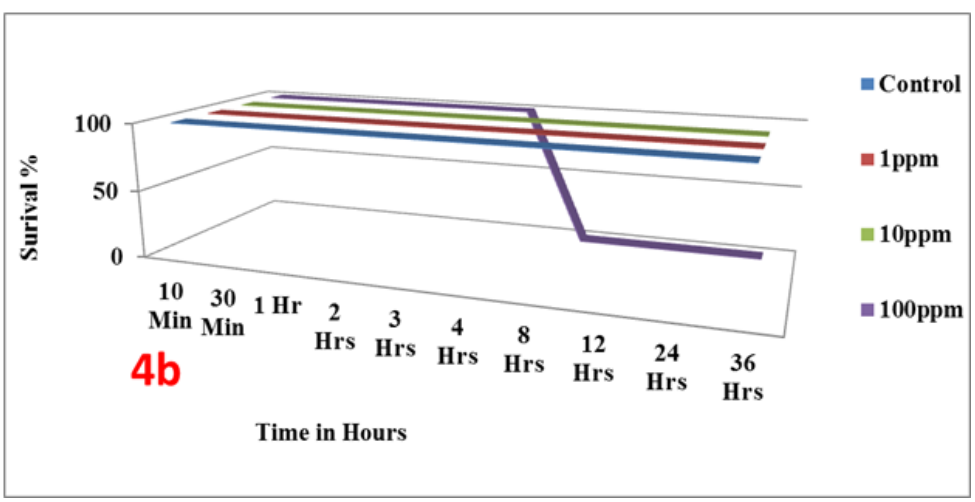
OL-2 &12

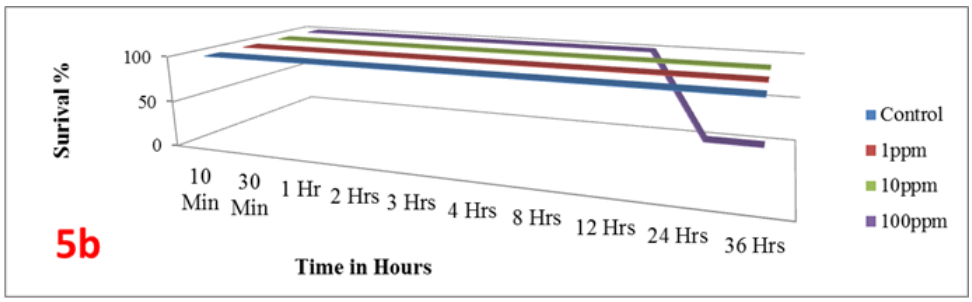
NEMATICIDAL ACTIVITIES OF CHEMICAL ENTITIES/ SYNTHETIC CHEMICALS

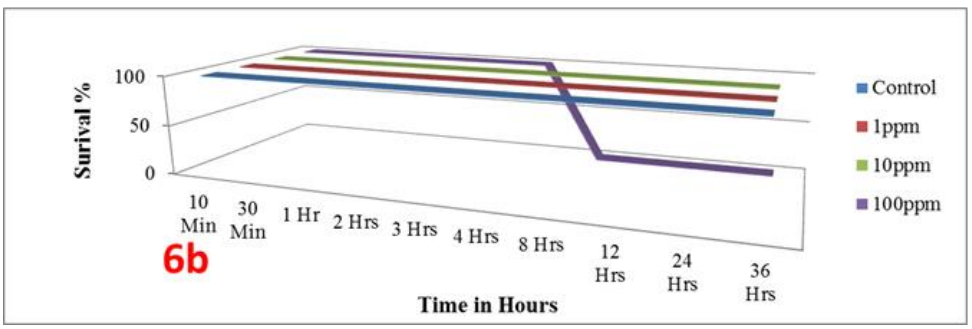
While many synthetic chemicals and orange oil and cinnamon oil showed the lowest survivability efficacy against C. elegans and mortality in root knot nematode, in most cases complete mortality was observed at higher concentration and a time lapse of a few hours.

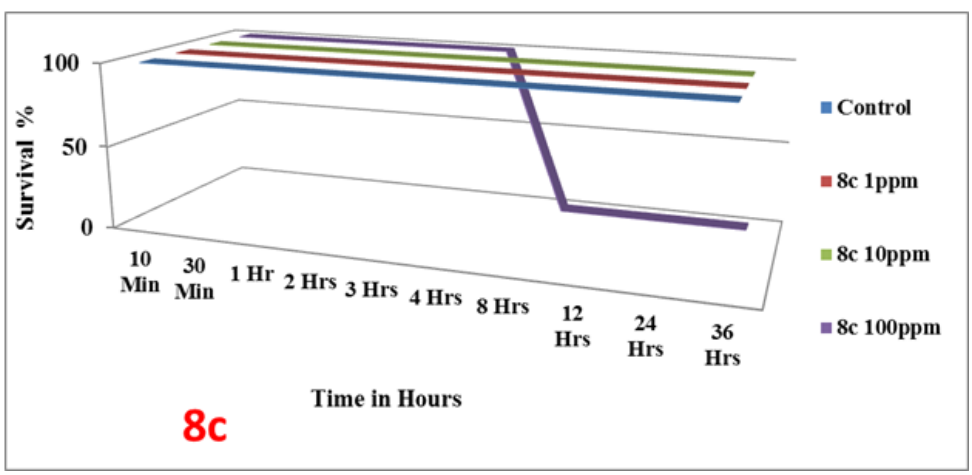

100
80
60
40
20
0
Surival %
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Contr ol
1ppm
10ppm
100pp m
4a

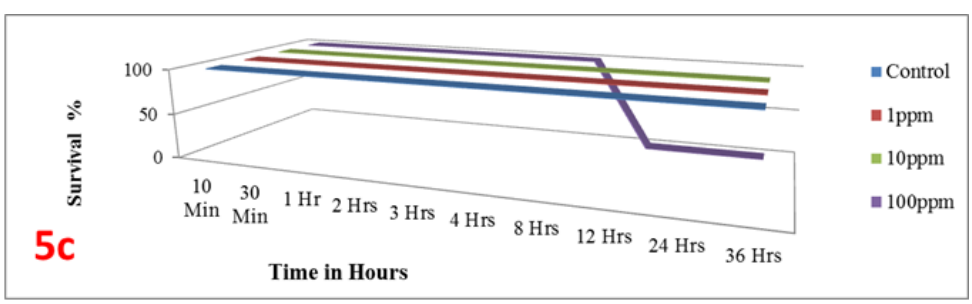

100
80
60
40
20
0
Surival %
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Control
1ppm
10ppm
100pp m

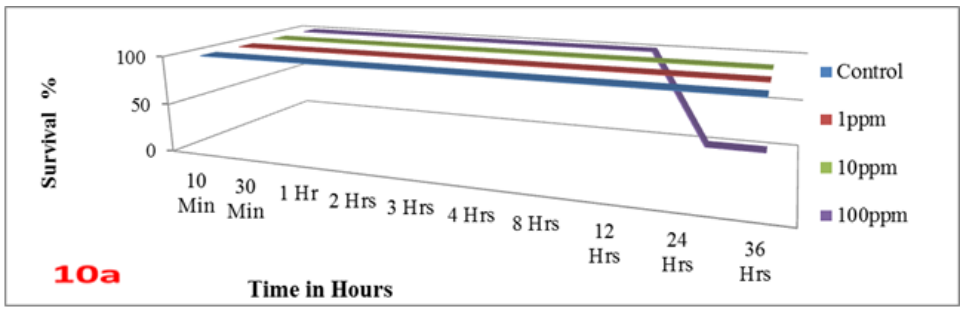

100
50
0
Surival %
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Control
1ppm
10ppm
100ppm
4b

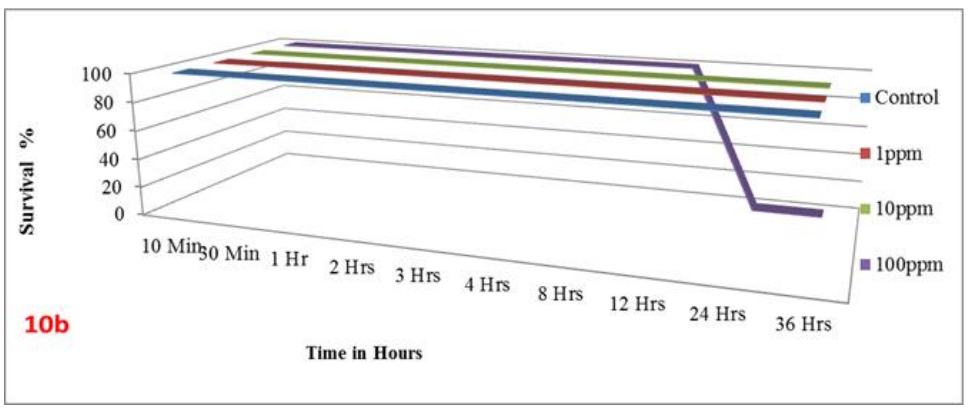

Surival %
100
50
0
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Control
1ppm
10ppm
100ppm
5b

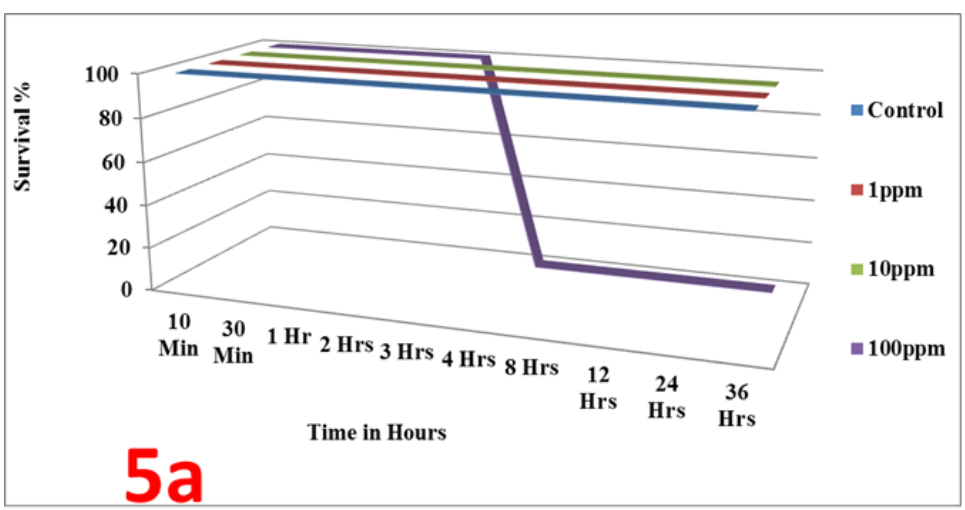

Surival %
100
50
0
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Control
1ppm
10ppm
100ppm
6b


Survival %
100
50
0
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Control
8c 1ppm
8c 10ppm
8c 100ppm
8c


Survival %
100
50
0
10 Min
30 Min
1 Hr
2 Hrs
3 Hrs
4 Hrs
8 Hrs
12 Hrs
24 Hrs
36 Hrs
Time in Hours
Control
1ppm
10ppm
100ppm
5c

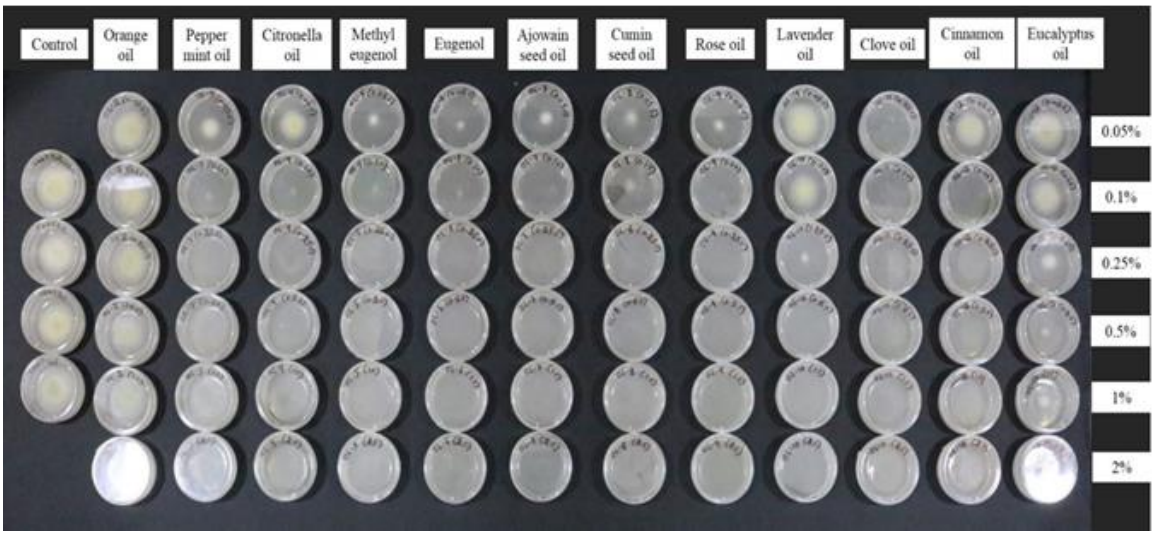


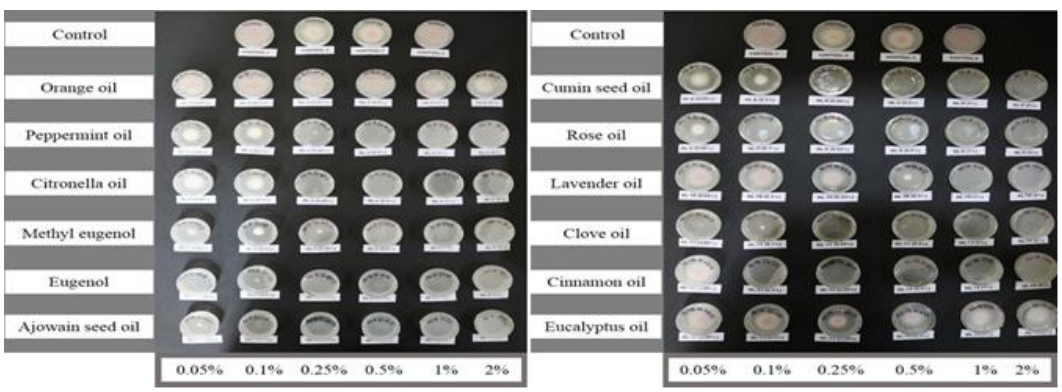




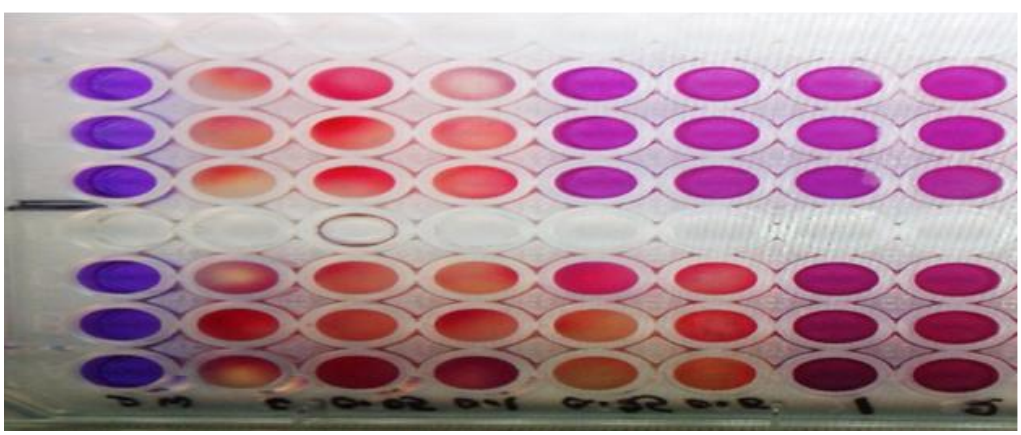
Screening for the fungicidal activity

Among the essential oil extracts screened with Food Poison Technique, inhibition of mycelial growth was found be significant with orange oil and cinnamon oil.





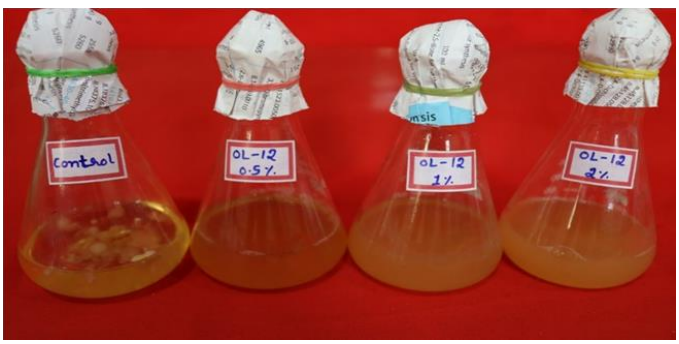
Similar results were observed even under Resazurin Assay method.



Pink/Colorless: Live Purple: Dead

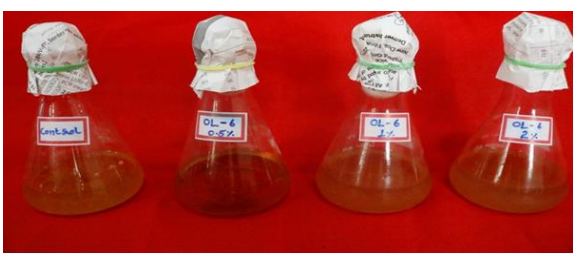
Scanning for Activity against *Aspegillus flavus*

Eugenol **Ajowain seed oil**



Rose oil **Cinnamon oil**

Scanning for activity against *Rhizoctonia bataticola*



Eugenol **Ajowain seed oil**

REFLECTIONS AND CONCLUSIONS

Meloidogyne incognetta nematode infects all vegetables, tubers and flower crops. This nematode causes a severe damage and loss attributed to its infestation ranges from 60 to 100% crop loss. Hence controlling of this nematode is challenging. The results of screening a few essential oils showed that they inhibited the nematode In-vitro at 1% concentration on exposure within an hour of incubation and further the combination of different essential oils provided the effective control within 30 minutes of exposure and these tests were confirmed with model system *Caenorhabditis elegans* and further with M*eloidogyne incognetta*, the root knot causing nematode. The studies indicated that control of the nematode is possible by further formulating these essential oils and testing in pot and field conditions.

Further, studies are required to bring out the best combination of synthetic and organic based formulation to control the nematode population in the field as the seven chemical compounds, two essential oils showed the effectiveness in controlling nematodes at 10ppm concentrations.

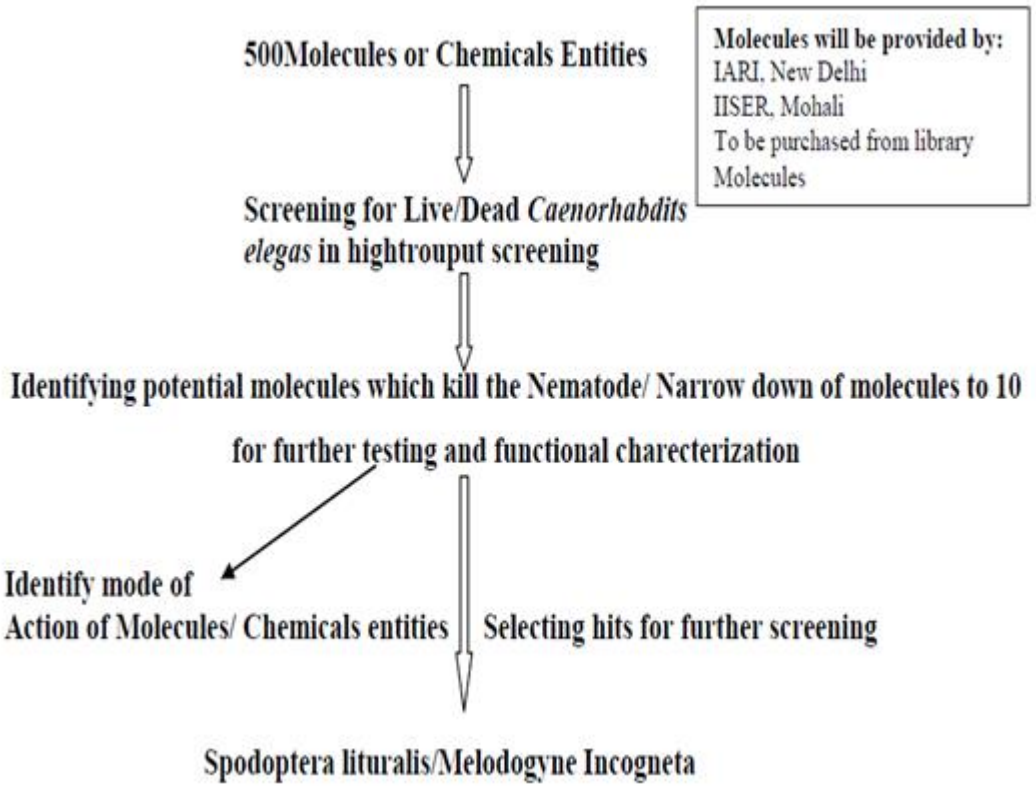
The studies will help the farming community by finding new solutions to control the disease causing nematodes and avoid loss of crop. It will also help in floriculture sector, horticultural crops and fruit crops by controlling the nematode infestation resulting in better yields and profit to farmers.

The screening for the fungicidal activity with essential oil was evaluated by microtiter method, food poison method, pyocynin assay and further confirmed by the Resazurin assay for controlling cell disruption there by controlling the fungal inoculum. Further testing in pot culture and field evaluation needs to be carried out.

Efficacy in field studies:

With good results in *in vitro* studies, experiments two formulations with the concentrations ranging from 0.5 to 10% were done by spraying in a small scale field trial to evaluate their performance in Chilli and Ber. During the study phytotoxicity was observed above 5% concentration of the spray. It was found that the formulations were able to control the powdery mildew of Ber and Chilli to the tune of 70%. These formulations are organic in nature. Hence, ease on environment, environment friendly, farmer friendly, do not cause any pollution and resistance development will not be possible as these essential oil and formulations will be having more than 30 compounds which will be acting simultaneously. Hence, longer utility and profitability to the farmers and farming community can be assured. This formulation is under field test in second year of evaluation on various vegetables and fruit crops. This formulation will boost the farmer's economy and saves the crop from loss on account of powdery mildew which is a major disease in chilli and other vegetables. This formulation also increases productivity by enhancing, retaining the chlorophyll for longer periods as the crop is protected from the powdery mildew and the fruit bearing capacity and size of the fruit is enhanced in these sprays.

During the implementation of the project, it has been possible to standardize the screening protocol with the help of Highthroughput screening using *Caenorhabdits elegans* as model nematode. Potential molecules which control the nematodes have been identified and narrowed down for further testing and functional characterization. Identifying the mode of action of molecules and selecting hits for further screening will have to be carried out.



Before spraying **After spraying**

ACTION POINTS

1. The project has been well conceived and systematically executed and has been successful in examining whether the screening procedure based on 96 well plate and utility of imaging and other methodologies adopted is able to make the screening procedure faster, cheaper and in timely fashion. This will help in screening molecules for action against insect pests and diseases.
2. As a future step arising out of the project, identifying the mode of action of molecules and selecting hits for further screening will have to be carried out.
3. Imaging of *Caenorhabdits elegans* using SPIM, Light Sheet Microscopy or Spinning Disk Confocal with Zyla 4.2 Plus and Sona sCMOS cameras has helped in high resolution imaging performance and Sona is the most sensitive sCMOS camera with a very large field of view, superior longevity and quantitative accuracy.
4. Molecular targets need to be established.
5. Testing formulations to control fungal and nematode in pot cultures and field conditions needs to be carried forward for *Aspergillus flavus, Fusarium oxysporum uduum* and *Rhizoctonia bataticola*. No clear description about major achievemets on anti parasitic molecule / extracts from highthroughput screening have emerged. The report highlighted that, very good antifungal activity against one or the other *Aspergillus* species tested. But to find utility of the above, more quantitative results are required. Otherwise, extending the same to practical application is difficult.
6. While several essential oils and plant extracts have been evaluated for activity against biological targets via the use of automation, miniaturized assays and large scale data analysis, there is need to identify the molecules responsible for effective control, mode of action and comparative costs to study the economics of disease management.
7. The plant extracts obtained from Jain University is not even partially characterized. Further, the synthetic chemicals from Kuvempu university is not explained in detail about the chemistry and properties. The report is not providing more comprehensive quantitative result of potential individual essential oil/ extract/ synthetic chemicals with reference to any of the leading reference standards.
8. Specific role of the essential oils, plant extracts and synthetic chemicals are not well presented. Therefore, it is very difficult to draw any specific conclusion about the beneficial role of above chemicals, extracts, synthetic chemicals in comparison with a reference standard and their practical utility.
9. The infrastructural facilities created at the centre was used by three masters students for their research work. Two projects have been received from Department of Science and Technology, Government of India which will be taken up by using the facilities created by the project.

10. The facilities are also being used by students from various disciplines such as nematology, dental pathology, microbiology and biotechnology from different institutes. It is recommended that the facilities should be exclusively used for the development of agriculture by taking up more research in the field. If at all the facilities are spared, it should be on revenue generation method since the techniques involve expenditure.
11. Patenting of process of organic chemical efficiency may be initiated.
12. The economics of the technology may be worked out.

RESEARCHABLE ISSUES

5. Relevance of secondary metabolites such as alkaloids, phenols and terpenoids which have both beneficial and harmful effects on insect control with reference to their control needs to be documented along with economics.
6. Development of standard protocols for screening compounds for biological targets is needed (especially for fungal diseases, nematodes and bacteria).
7. Need for efficiency and efficacy of plant metabolites in critical limits on pathogens under varied climatic conditions (i.e., temperature, rainfall and relative humidity).
8. Need for more basic and applied research work on hormones/ pheromones, i.e., phytochemical changes in plants due to climate change.

REFERENCES

Adjou, E. S., Kouton, S., Dahouenon-Ahoussi, E., Sohounhloue, C. K. and Soumanon, M., 2012, Antifungal activity of Ocimum canum Essential oil against Toxinogenic Fungi isolated from Peanut Seeds in post-harvest in Benin. *I. Res. J. Biological Sci.*, 1(7): 20-26.

Ahmad, N., Alam, M. K., Shehbaz, A., Khan, A., Mannan, A., Rashid, H. S., Bisht, D. and Owais, M., 2005, Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. *J. of Drug Target,* 13: 555-561.

Ajith Kumar, K., Naik, M. K., Allolli, T. B. and Hosamani, R. M., 2005, Evaluation of genotypes, fungicides and plant extracts for the management of aflatoxin contamination in chilli caused by Aspergillus flavus. *Indian. J. Pl. Prot.*, 33 (1): 115-118.

Al-Omair, A. and Helaleh, M. T. H., 2004, Selected-ion-storage Gas Chromatography Mass Spectrometry analysis of polcyclic aromatic hydrocarbons in palm dates and tuna fish. *Chromatogahia,* 59: 715-719.

Amaike, S. and Nancy, P. K., 2011, Aspergillus flavus. *Annual Review of Phytopathology*, 49: 107-133.

Amoabeng BW, Gurr GM, Gitau CW, Stevenson PC, 2014, Cost:benefit analysis of botanical insecticide use in cabbage: implications for smallholder farmers in developing countries. *Crop Prot* 57:71– 76. https://doi.org/10.1016/j.cropro.2013.11.019

Ansari, M. M., 1995, Control of sheath blight of rice by plant extract. *Indian Phytopathol*., 48(3): 268-270.

Avila-Sosa, R., Gastelum-Reynoso, G., Garcia-Juarez, M., Cruz, M. S. M., Navarro-Cruz, A. R. and Davila-Marquez, R. M., 2011, Evaluation of different Mexican plant extracts to control anthracnose, *Food and Bioprocess Technology,* 4: 655-659.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M., 2008, Biological effects of essential oils - A review. *Food Chem. Toxicol.*, 46: 446-475.

Baldev, B., Ramanujam, S. and Jain, H. K., 1988, Pulse crops. Fungal diseases of pulse crops. *Pulse crops*, 2: 266-267.

Bansal, R. K. and Sobti, A. K., 1990, An economic remedy for the control of two species of Aspergillus on groundnut. *Indian Phytopathol*., 43: 451-452.

Barzman M, Barberi P, Birch ANE, Boonekamp P, Dachbrodt-Saaydeh S, Graf B, Hommel B, Jensen JE, Kiss J, Kudsk P, Lamichhane JR, Messean A, Moonen AC, Ratnadass A, Ricci P, Sarah JL, Sattin M, 2015, Eight principles of integrated pest management. *Agron Sustain Dev* 35:1199–1215.

Belmain SR, Amoah BA, Nyirenda SP, Kamanula JF, Stevenson PC, 2012, Highly variable insect control efficacy of Tephrosia vogelii chemotypes. *J Agric Food Chem* 60:10055–10063.

Beuchat, L. R. Y. and Golden, D. A., 1989, Antimicrobials occurring naturally in foods. *Food Technol.,* 43: 134-142.

Bhaskar, A. V., Rahman, M. A. and Rao, K. C. S., 2005, Occurrence and Management of dry corm rot diseases (Rhizoctonia solani) in amorphophallus. *Annual of biology*, 21(2): 231-240.

Bigalke, D. L., 1984, Methods used for monitoring the microbiological quality of raw milk. *Dairy Food Sanit.* 4:189-190.

Bluma, R. V., Amaiden, M. R., Daghero, J. and Etcheverry, M., 2008, Control of Aspergillus section Flavi growth and aflatoxin accumulation by plant essential oils. *J. Appl. Microbiol*,, 105: 203-214.

Bonnier., Mark, K., Tomasz, P., Wrobel., Katarsyna, M., Malgorzata, B., Amaya, G., Alfonso, B., and Hugh, B., 2015, Cell viability assessment using the Alamar blue assay: A comparison of 2D and 3D cell culture models. *Toxicol. in Vitro*, 29: 124-131.

Borel B, 2017, CRISPR, microbes and more are joining the war against crop killers. *Nature* 543:302–304.

Bratman S. and Girman. 2003. A Hand book of Herbs and Supplement and their Therapeutic Uses. St. Louis: Mosby, 769-771.

Burt, S. A. and Reinders, R. D., 2003, Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. *Lett. Appl. Microbiol.*, 36: 162-167.

Burt, S., 2004, Essential oils: their antibacterial properties and potential applications in foods-A review. *Int J. Food Microbiol*., 94: 223-253.

Caralt, J. S., Uzarski, S., Iacob, K. P., Obergfell, N., Berg, B. M., Bijonowski, K. M., Kiefer, H. H., Ward, A., Wandinger-Ness, W. M., Miller, Z. J. Zhang., M. M. Abecassis. and J. A. Wertheim., 2015, Optimization and critical evaluation of decellularization strategies to develop renal extracellular matrix scaffolds as biological templates for organ engineering and transplantation. *American J. of Transplantation*, 15(1): 64-75.

Carson, C., Hammer, K. A. and Riley, T. V., 2006, Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiol. Review*, 19: 50-62.

Chagnon M, Kreutzweiser D, Mitchell EA, Morrissey CA, Noome DA, Van der Sluijs JP, 2015, Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res Int* 22:119–134. https://doi.org/10.1007/ s1135 6-014-3277-x

Chaieb, K., Zmantar, T., Ksouri, R., Hajlaoui, H., Mahdouani, K., Abdelly, C. and Bakhrouf, A., 2007, Antioxidant properties of essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. *Mycosis*, 50: 403-406.

Chami, F., Chami, N., Bennis, S., Trouillas, J. and Remmal, A., 2004, Evaluation of carvacrol and eugenol as prophylaxis and treatment of vaginal candidiasis in an immunosuppressed rat model. *J. Antimicro. Chemother*, 54: 909-914.

Chatterjee, D., 1990, Inhibition of fungal growth and infection in maize grains by spice oils *Lett. Appl. Microbiol.,* 11:148-151.

Chipley, J. R. and Uraih, N., 1980, Inhibition of Aspergillus growth and aflatoxin release by derivatives of benzoic acid. *Appl. Environ. Microbiol*., 40:352-357.

Cotty, P. J., 1989, Virulence and cultural characteristics of two Aspergillus flavus strains pathogenic on cotton. *Phytopathol.*, 79: 808-814.

Cox, S. D., Mann, C. M., Markham, J. L., Gustafson, J. E., Warmington, J. R. and Wyllie, S. G., 2001, Determining the antimicrobial actions of tea tree oil. *Molecules,* 6: 87-91.

Cuellar-Cruz, M., Castano, I., Arroyo-Helguera, O. and Penas., 2009, Oxidative stress response to menadione and cumene hydroperoxide in the opportunistic fungal pathogen Candida glabrata. *Mem Inst Oswaldo Cruz*, 104(4): 649-654.

Desneux N, Decourtye A, Delpuech JM, 2007, The sublethal efects of pesticides on benefcial arthropods. *Annu Rev Entomol* 52:81– 106.

Dhingra, O. D. and Sinclair, J. B., 1994, Basic plant Pathologt Methods. CRS press, London., Pp:443.

Diener, U. L., Cole, R. J., Sanders, T. H., Payne, G. A., Lee, L. S. and Klich, M. A., 1987, Epidemiology of aflatoxin formation by Aspergillus flavus. *Annual review of Phytopathol*., 25: 249-270.

Dubey N, Shukla R, Kumar A, Singh P, Prakash B, 2011, Global scenario on the application of natural products in integrated pest management programmes. In: Dubey NK (ed) Natural products in plant pest management. CAB International, Wallingford, pp 1–20

Duke J., and M. Bogenschutz, 2002, Duke Hand book of medicinal Plants. 2nd Boca Raton, FL: CRC Press; 529.

Edris, A. E., 2007, Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: *A review. Phytother.*, 21: 308-323.

Eddleston M, Karalliedde L, Buckley N, Fernando R, Hutchinson G, Isbister G, Konradsen F, Murray D, Piola JC, Senanayake N, Sherif R, Singh S, Siwach SB, Smit L, 2002, Pesticide poisoning in the developing world—a minimum pesticides list. *Lancet* 360:1163–1167

Elshafie, H. S., Mancini, E., Camele, I., De Martino, L. and De Feo, V., 2015, In vivo antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. *Ind. Crops Prod.,* 66: 11-15.

Essar, D. W., Eberly, L., Hadero, A. and Crawford, I., 1990, Identification and characterization of genes for a second anthranilate synthase in Pseudomonas aeruginosa: Interchangeability of the two anthranilate synthases and evolutionary implications. *J. Bacteriol*., 172: 884-900.

Fengfeng, W., Yamei, J., Xueming, X. and Na, Y., 2017, Electro fluidic pretreatment for enhancing essential oil extraction from citrus fruit peel waste. *J. Clean. Prod.*, 159: 85-94.

Fernanda, C., da Silva., Sara, M., Chalfoun., Virgínia, M., de Siqueira., Deila, M., dos S. Botelho., Nelson Lima. and Luis R. Batista., 2012, Evaluation of antifungal activity of essential oils against potentially mycotoxigenic Aspergillus flavus and A. parasiticus. *Rev. bras. farmacogn.*, 22: 102-206.

Fitzgerald, D. J., Stratford, M., Gasson, M. J., Ueckert, J., Bos, A. and Narbad, A., 2004, Mode of antimicrobial action of vanilla against Escherichia coli. Lactobacillus plantarum and Listeria innocua. *J. of Applied Microbiology*, 97: 104-113.

Frank, L. H. and DeMoss, R. D., 1959, On the biosynthesis of pyocyanine. *J. Bacteriol.*, 77(6): 776-782.

Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R. and Nazzaro, F., 2010, Preservation of chicken breast meat treated with thyme and balm essential oils. *J. Food Sci.,* 75: 528-535.

Gaalen, J. D., de Bruijn, C. E., Wilson, C. A., van Blitterswijk, A. J., Verbout, J., Alblas, W. J. and Dhert., 2010, Relating cell proliferation to in vivo bone formation in porous Ca/P scaffolds, *J. Biomed Mater Res.,* 92(1): 303-10.

Gayoso, C. W., Lima, E. O., Oliveira, V. T., Pereira, F. O., Souza, E. L., Lima, I. O. and Navarro, D. F., 2005, Sensitivity of fungi isolated from onychomycosis to Eugenia cariophyllata essential oil and eugenol. *Fitoterapia*, 76: 247-249.

Ghalem, B. R., 2016, Essential oils as antimicrobial agents against some important plant pathogenic bacteria and fungi. Plant-Microbe Interaction: *An Approach to Sustainable Agriculture*, 2: 271-296

Gloeckner, T., Jonuleit, H. D. and Lemke., 2001, Monitoring of cell viability and cell growth in a hollow-fiber bioreactor by use of the dye Alamar Blue, *J. Immunol. Methods*, 252(12): 131- 8.

Grover, R. K. and Sukhuja, P. K. 1981, Some pathological studies on Rhizoctonia bataticola leaf bight of mungbean. *Ind. Phytopathol.,* 34: 24-29.

Gupta, V., Govindaiah. and Datta, R. K., 1996, Plant extracts; a non-chemical approach to control Fusarium diseases of mulberry. *Curr Sci.* 71(5):406-409.

Gurr GM, Wratten SD, Landis DA, You M, 2017, Habitat management to suppress pest populations: progress and prospects. *Annu Rev Entomol* 62:91–109.

Haware, M. P., 1990, Fusarium wilt and other important diseases of chickpea in the Mediterranean area. *Options mediterraneennes-serie seminairies*, 9: 61-64.

Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P. and Denning, D. W., 2007, Aspergillus flavus: human pathogen, allergen and mycotoxin producer. *Microbiol.,* 153, 1677-1692.

Horn, B. W., Moore, G. G. and Ignazio Carbone., 2009, Sexual reproduction in Aspergillus flavus. *Mycologia,* 101 (3): 423-429.

Hu, Y., Zhang, J., Kong, W., Zhao, G. and Yang, M., 2007, Mechanisms of antifungal and antiaflatoxigenic properties of essential oil derived from turmeric (Curcuma longa L.) on Aspergillus flavus. *Food Chem.*, 220: 1-8.

Hyldgaard, M., Mygind, T. and Rikke, L. M., 2012, Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Front. Microbiol.*, 3: 1-24.

Ikeura, H. N., Somsak, F., Kobayashi, S., Kanlayanarat. and Hayata, Y., 2011, Application of selected plant extracts to inhibit growth of Penicillium expansum on Apple fruits. *Plant Pathol. J*., 10: 79-84.

Isman MB,1997 Neem and other botanical insecticides: barriers to commercialization. *Phytoparasitica* 25:339–344.

Isman MB, 2000, Plant essential oils for pest and disease management. *Crop Prot* 19:603–608.

Isman MB, 2006, Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* 51:45–66.

Isman MB, 2008, Botanical insecticides: for richer, for poorer. *Pest Manag Sci* 64:8–11.

Isman MB, 2017, Bridging the gap: moving botanical insecticides from the laboratory to the farm. *Ind Crops Prod* 110:10–14.

Isman MB, Grieneisen ML, 2014, Botanical insecticide research: many publications, limited useful data. *Trends Plant Sci* 19:140–145

Jabar, M. A. and AL-Mossanawi, A., 2007, Susceptibility of some multiple resistant bacterial to garlic extract. *African J. of Biotechnol.,* 6: 771-776.

Jay, J. M. and Rivers, G. M., 1984, Antimicrobial activity of some food flavoring compounds. *J. of Food Safety,* 6: 129-139.

Jayaratne, K. H. T., Paranagama, P. A., Abeywickrama, K. P. and Nugaliyadde, L., 2002, Inhibition of Aspergillus flavus Link and Anatoxin Formation by Essential Oils of Cinnamomum zeylanicum (L.) and Cymbopogon nardus Rendle. *Tropical Agricultural Research,* 14: 148-153.

Joseph, G. S., Jayaprakasha, G. K., Selvi, A. T., Jena, B. S. and Sakariah, K. K., 2005, Anti-aflatoxigenic and antioxidant activities of Garcinia extracts. *Int. J. Food Microbiol.*, 101: 153-160.

Kalemba, D. and Kunicka, A., 2003, Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.,* 10: 813-829.

Kanan, G. J. and AL-Najar, R. A., 2008, In vitro antifungal activities of various plant crude extracts and fractions against citrus post-harvest disease agent Penicillium digitatum. *J. of Biological Science,* 1: 89-99.

Kaushal, G., Rao, P. B. and Chauhan, S. V. S., 2003, Efficacy of some botanicals of the family compositae against Rhizoctonia solani khun. *Indian J. Mycol. Plant Pathol*., 33(2): 230-235.

Kedia, A., Prakash, B., Mishra, P.K., Dubey, N. K., 2014, Antifungal and anti-aflatoxigenic properties of Cuminum cyminum (L.) seed essential oil and its efficacy as a preservative in stored commodities. *International J. of Food Microbiol.*, 168-169: 1-7.

Khalid, I. and Ilyas, M. B., 2000, Screening of chickpea germplasm against dry root rot disease (Macrophomina phaseolina) in pots/glass house. *Pakistan J. of Phytopathol.*, 12: 66-70.

Khan, S. A., 1999, Control of plant pathogens by plant products preparations. M.Sc. Thesis, Dept. of Plant Pathology, Agric. Univ., Udaipur..

Khan ZR, Midega CA, Pittchar J, Bruce TJ, Pickett JA, 2012, 'Push– Pull' revisited: The process of successful deployment of a chemical ecology based pest management tool. In: Gurr GM, Wratten SD, Synder WE, Read DMY (eds) Biodiversity and insect pests: key issues for sustainable management. Wiley, *Chicheste*, pp 259–275

Kim, J. H., Campbell, B. C., Yu, J., Mahoney, N. E., Chan, K. L., Molyneux, R. J., Bhatnagar, D. and Cleveland T. E., 2005, Examination of fungal stress response genes using Saccharomyces cerevisiae as a model sys- 706 / Molecular Plant-Microbe Interactions tem: Targeting genes affecting aflatoxin biosynthesis by Aspergillus flavus Link. *Appl. Microbiol. Biotechnol.*, 67:807-815.

Kim, J. H., Mahoney, N. E., Chan, K. L., Molyneux, R. J. and Campbell, B. C., 2006, Controlling food-contaminating fungi by targeting their antioxidative stress-response system with natural phenolic compounds. *Appl. Microbiol. Biotechnol*., 70:735-739.

Kishore, N. N., Dubey, K., Tripathi, R. D., and Singh, S. K., 1982, Fungitoxicity of the leaf extract of the some higher plants against some fungi. *Natr. Academic Science,* 5(2): 3-45.

Klich, M. A., 2007, Aspergillus flavus: the major producer of aflatoxin. *Mol. Plant Pathol*., 8: 713-722.

Koul O, 2004, Insect antifeedants. CRC, Boca Raton

Koul O, Walia S, Dhaliwal G, 2008, Essential oils as green pesticides: potential and constraints. *Biopestic Int* 4:63–84

Kumar, A., Shukla, R., Singh, P., Prasad, C. S. and Dubey, N. K., 2008, Assessment of Thymus vulgaris L. essential oil as a safe botanical preservative against post-harvest fungal infestation of food commodities. *Innovat. Food Sci Emerg.*, 9: 575-580.

Kumar, S., Sharma, S., Pathak, D. V. and Beniwal, J., 2011, Integrated management of Jatropa root rot caused by Rhizoctonia bataticola. *J. Tropi. Fore. Sci.*, 23(1): 35-41.

Lang, G. and Buchbauer, G., 2012, A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. *Flavour Fragr.* J., 27: 13-39.

Latif. and Faten, M., 2016, Postharvest Application of Some Essential Oils for Controlling Gray and Blue Moulds of Apple Fruits. *Plant pathol. J.,* 2: 1812-5387.

Leo, P., Hoffman, P. S., Malcolm, G. B., Benson, R. F. and Keen, M. G., 1984, Determination of catalase, peroxidase, and superoxide dismutase within the genus Legionella. *J. of Clinical Microbiol.*, 20(3): 421-429.

Li H, Cheng F, Wei Y, Lydy MJ, You J, 2017, Global occurrence of pyrethroid insecticides in sediment and the associated toxicological efects on benthic invertebrates: an overview. *J Hazard Mater* 324:258–271

Lopez, P., Sanchez, C., Batlle, R. and Nerin, C., 2005, Solid- and Vapor-phase antimicrobial activities of six essential oils: susceptibility of selected food borne bacterial and fungal strains. *J. of Agri. Food Chem.*, 53: 6939-6946.

Lopez-Reyes, J. G., Spadaro, D., Preile, A., Garibaldi, A. and Gullino, M. L., 2013, Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo. *J. Food Protect.*, 76: 631–639.

Lubinsky, P., Bory, S., Hernandez, H., Juan, K., Seung, C. and Gomez-Pompa, A., 2008, Origins and Dispersal of Cultivated Vanilla (Vanilla planifolia Jacks. [Orchidaceae]). *Economic Botany,* 62(2): 127-138.

Macwan, S. R., Dabhi, B. K., Aparnathi, K. D. and Prajapati, J. B., 2016, Essential oils of herbs and spices: Their antimicrobial activity and application in preservation of foods. *Int. J. Curr. Microbiol. Appl. Sci.*, 5: 885-901.

Malik, E. P. and Singh, M. B., 1980, Plant Enzymology and Hittoenzymology. Report and Opinion, 4: 8-35.

Maren, A. K., 2007, Aspergillus flavus: the major producer of aflatoxin. *Mol. Plant Pathol.,* 8(6): 712-722.

Martin, M. G. and John, M. Wagacha., 2017, Antifungal Activity of Essential Oil of Eucalyptus camaldulensis Dehnh. Against Selected Fusarium spp. *Int. J. of Microbiol.,* 2017: 1-7.

Masang, P., 2005, Cleaner production of essential oils by steam distillation. *J. Clean. Prod.*, 13: 833-839.

McNicholl, B. P., McGrath, J. W. and Quinn, J. P., 2006, Development and application of a resazurin-based biomass activity test for activated sludge plant management. *Water Res*., 41:127-133.

Michalek., Kinga, S., Piotr, L., Anna, M. and Juliusz, P., 2010, Contents of microscopic fungi in dusts coming from cereal analysis laboratories, *Ann Agric Environ Med*, 17, 101-106.

Michelle, M., Manita, G., Svetlana, A., Sarkisova. and Marianna, A. P., 2016, PyocyaninnExtraction and Quantitative Analysis in Swarming Pseudomonasn aeruginosa. *Bio-protocol*, 6: 1-10.

Mijat Bozovic, M., Garzoli, S., Sabatino, M., Pepi, F., Baldisserotto, A., Andreotti, E., Romagnoli, C., Mai, A., Manfredini, S. and Ragno, R., 2017, Essential oil extraction, chemical analysis and anti-Candida activity of Calamintha nepeta (L.) Savi subsp. glandulosa Ball-New Approaches. *Molecules,* 22: 203.

Mishra, M. and Tiwari, S. N., 1992, Toxicity of Polyathia uongifolia against fungal pathogen of rice. *Ind. Phytopathol.,* 45: 59-61.

Mondali, N. K., Mojumdar, A., Chatterje, S. K., Banerjee, A., Datta, J. K. and Gupta, S., 2009, Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species in vitro culture medium. *J. of Appl. Sci. Env. Manag.,* 13: 49-53.

Montes-Belmont, R. and M. Carvajal., 1998, Control of Aspergillus flavus in maize with plant essential oils and their components. *J. Food Protect,* 61: 616-619.

Narumol, M. N. and Jantamas, S., 2014, Efficacy of Clove oil in the inactivation of Aspergillus flavus on peanuts. *Acta Horticulture,* 1023: 301- 306.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R. and De Feo, V., 2013, Effects of essential oils on pathogenic bacteria. *Pharmaceuticals,* 6: 1451-1474.

Nazzaro, F., Fratianni, F., Coppola, R. and De Feo, V., 2017, Essential Oils and Antifungal Activity. *Pharmaceuticals (Basel)*. 10(4): 86.

Nene, Y. L. and Thapliyal, P. N., 1993, Fungicides in Plant Disease Control. Oxford and IBH Publishing Co., New Delhi, India 579 pp.

Nene, Y. L., 1996. Surpala's Vrikshayurveda. *Agri-History Bulletin* 1:80-87.

Nene, Y. L., Haware, M. P. and Reddy, M. V., 1978, Diagnosis of some wilt like disorders of chickpea (Cicer arietinum L.). *Information Bulletin No. 3, ICRISAT*. p. 44.

Nguefack, J., Leth, V., Amvam Zollo, P. H. and Mathur, S. B., 2004, Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *Int. J. Food Microbiol.*, 94: 329-334.

Obagwu, J., 2003, Developing bio pesticides for control of citrus fruit pathogens of importance in global trade. PhD Thesis, University of Pretoria, South Africa.

Paranagama, P. A., 1991, Analysis of Sri Lankan essential oils by gas chromatography and mass spectroscopy. Industrial Technology Institute, Colombo, Sri Lanka.

Paranagama, P., Abeyasekara, K. H., Abeywickrama, K. and Nugaliyadde, L., 2003, Fungicidal and anti-aflatoxigenic effects of the essential oil of Cymbopogon citratus (DC.) Stapf. Against Aspergillus flavus isolated from stored rice. *Lett. Appl. Microbiol.*, 37(1): 86-90.

Passone, M. A., Natalia, S., Girardi, C. A. and Ferrand, M. E., 2012, In vitro evaluation of five essential oils as botanical fungi toxicants for the protection of stored peanuts from Aspergillus flavus and A. parasiticus contamination. Int. Bio deterioration and *Biodegradation,* 70: 82-88.

Pawar, V. C. and V. S. Thaker., 2006, In vitro efficacy of 75 essential oils against Aspergillus niger. *Mycoses,* 49: 316-323.

Pereira, M. C., Chalfoun, S. M., Pimenta, C. J., Angelico, C. L. and Maciel, W. P., 2006, Spices, fungi mycelial development and ochratoxin A production. *Science Resource Essays,* 1: 38-42.

Prakash, B., Singh, P., Kedia, A. and Dubey, N. K., 2012, Assessment of some essential oils as food preservatives based on antifungal, anti-aflatoxin, antioxidant activities and in vivo efficacy in food system. *Food Research Int.*, 49: 201-208

Pretty J, Benton TG, Bharucha ZP, Dicks LV, Flora CB, Godfray HCJ, Goulson D, Hartley S, Lampkin N, Morris C, Pierzynski G, Prasad PVV, Reganold J, Rockstrom J, Smith P, Thorne P, Wratten S, 2018, Global assessment of agricultural system redesign for sustainable intensifcation. *Nature Sustain* 1:441–446.

Rangaswamy, G., 1996, Diseases of crop plants. *Appl. Environ. Microbiol,* 70: 1836-1842.

Ravindran, P. and Balachandran, I., 2016, Under-utilized medicinal spices II, *Spice India.* 17: 32-36.

Razzaghi-Abyaneh, M. M., Shams-Ghahfarokhi, M. B., Rezaee, K., Jaimand, S., Alinezhad, R., Saberi, R. and Yoshinari, T., 2009, Chemical composition and antiaflatoxigenic activity of Carum carvi L., Thymus vulgaris and Citrus aurantifolia essential oils. *Food Control,* 20: 1018-1024.

Reddy, C. S., Reddy, K. R. N., Raja Kumar, N., Laha, G. S. and Muralidharan, K., 2004, Exploration of aflatoxin contamination and its management in rice. *J. Mycol. Pl. Pathol.*, 34(3): 816-820.

Ren, L. F., Tapias, B. J., Jank, D. J., Mathisen, M., Lanuti, H. C. and Ott., 2015, Ex vivo non-invasive assessment of cell viability and proliferation in bio-engineered whole organ constructs. *Biomaterials,* 52: 103-112.

Roat, B. L., 2000, Management of fruit rots of chilli by partially purified plant products preparation. M.Sc .. Thesis, Dept. of Plant Pathology, M.P. Univ. of Ag. & Tech., Udaipur.

Roberts, P., 1999, Rhizoctonia-forming fungi. *Royal Botanic Gardens*, 5: 239.

Rodriguez, C. J. O. and Velandia, M. J., 1998, Evaluation of extracts of 10 medicinal plants for their efficiency to control enthrones of curuba under laboratory conditions. *Fitopathogia Colombiana*, 21(2):73-78.

Sanecha, P. and Srivastava, H. P., 1982, Leaf blight of Vigna aconitifolia caused by Macrophomina phaseolina. *Ind. phytopathol*., 35: 160-161.

Satish, S., Mohana, D. C., Ranhavendra, M. P. and Raveesha, K. A., 2007, Antifungal activity of some plant extracts against important seed borne pathogens of Aspergillus sp. *J. Agril. Technol.*, 3(1): 109-119.

Shahnaz, D. S., Khaliq. and Marium, T., 2010, Comparative effect of plant extract of Datura alba Nees and Cynodon dactylon (L.) Pers., alone or in combination with microbial antagonists for the control of root rot disease of cowpea and okra. *Pak. J. Botany*, 42(2): 1273-1279.

Sharma, R. P., 2000, Management of fruit rots of tomato by plant products preparation. M.Sc. Thesis, Dept. of Plant Pathology, Ag. Univ., Udaipur.

Sharma, R. R., Goar, N. H. and Sharma, P., 2005, Effect of plant extract on growth of Rhizoctonia solani and development of maize. *Indian J. Mycol. Pl. Pathol*., 35(2): 377-379.

Sharville, E.G., 1960, The nature and use of modern fungicides. Burgess Publishing Co., Minnesota, U.S.A., pp. 3.

Shaon Kumar Das, 2016, Screening of Bioactive Compounds for Development of New Pesticides: A Mini Review, *Universal Journal of Agricultural Research* 4(1): 15-20, 2016

Shivpuri, A., Sharma, O. P. and Aria, S. L., 1997, Fungitoxic properties of plant extract against pathogenic fungi. *Indian J. Mycol. Pl. Pathol.,* 27: 29-35.

Shyamala, B. N., Madhava, N. M., Sulochanamma, G. and Srinivas, P., 2007, Studies on the antioxidant activities of natural vanilla extract and its constituent compounds through in vitro models. *J. of Agri. and Food Chem.*, 35: 7738-7743.

Simic, A., M. D. Sokovic., M. Ristic., S. Grujic-Jovanovic , J. Vukojevic. and P. D. Marin., 2004, The chemical composition of some lauraceae essential oils and their antifungal activities. *Phytother. Res.,* 18: 713-717.

Simko, P., 2005, Factors affecting elimination of polycyclic aromatic hydrocarbons from somoked meat foods and liquid smoke flavorings. *Molecule Nutrition Food Research,* 49: 637-647.

Sindhan, G. S., Hooda, I. and Parashar, R. D., 1999, Effect of some plant extracts on the vegetable growth of root rot causing fungi. *Indian J. Mycol. Pl. Pathol.,* 29(1): 110-111.

Singh, A. S. and Agarwal, S. N., 2002, Prevalence of chickpea wilt and dry root rot diseases in Vindhyan plateau zone of Madhya Pradesh. *Ind. J. of Pulses Research,* 15(2): 14-19.

Singh, S. D., 1999, Soil borne diseases of chickpea. *J. of Pl, Protec. Research,* 48(3): 348-354.

Singh, S. K., Nene, Y. L. and Reddy, M. V., 1990, Effect of age on susceptibility of chickpea to Rhizoctonia bataticola. *Int. Chickpea Newsletter,* 23: 25-26.

Singh, U. P., Singh, H. B. and Singh, R. B., 1980. The fungicidal effect of Neem (Azadiractha indica) in some soil borne pathogen of gram (Cicer arietinum). *Mycol.,* 72: 1077-1093.

Sinigagila, M., Reguly, M. L. A. and Aderade, H. H., 2004, Effect of vanilla on toxicant - indices mutation and mitotic recombination in proliferating somatic cells of Drosophilla melaogaster. *Environmental and Mutagenesis,* 11: 361-400.

Skinner, E. P., 1995, General anthropology division. *Anthropology News,* 36(3): 19.

Soliman, K. M. and Badea, B. I., 2002, Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food Chem. Toxicol.,* 40: 1669-1675.

Srichana, D., Phumruang, A. and Chongkid, B., 2009, Inhibition effect of betel leaf extract on the growth of Aspergillus flavus and Fusarium verticillioides. *International J. of Science Technol.*, 14: 74-77.

Srilakshmi, P., Sudini, H., Prasad, K. S., Devi, S. and Kumar, K. V. K., 2013, Investigations on bioactive secondary metabolites of Trichoderma spp. and their efficacy against aflatoxin contamination in groundnut. Recent advances in bio fertilizers and bio fungicides (PGPR) for sustainable agriculture. Proceedings of 3rd Asian Conference on Plant Growth Promoting Rhizobacteria (PGPR) and other Microbials, Manila, Philippines, 21-24 April, 350-358.

Srivastava, K. K, Gupta, P. K., Tripathi, Y. C. and Sarvate, R., 1997, Antifungal activity of plant products on spermoplane fungi of Azdardirachta indica, neem seeds. *Indian Forester,* 123: 157-161.

Sudha, S., Naik, M. K. and Ajithkumar, K., 2013, An integrated approach for the reduction of aflatoxin contamination in chilli (Capsicum annuum L.). *J. Food Sci. Technol.,* 50(1): 159-164.

Sumon KA, Ritika AK, Peeters E, Rashid H, Bosma RH, Rahman MS, Fatema MK, Van den Brink PJ, 2018, Efects of imidacloprid on the ecology of sub-tropical freshwater microcosms. *Environ Pollut* 236:432–441.

Tagoe, D., Baidoo, S., Dadzie, I., Kangah, V. and Nyarko, H., 2011, A comparison of the antimicrobial (antifungal) properties of onion (Allium cepa), Ginger (Zingiber officinal) and garlic (Allium sativum) on Aspergillus flavus, A. niger and Cladosporium herbarum using organic and water base extraction methods. *J. of Medicinal Plants*, 5: 281-287.

Tajkarimi, M. M., Ibrahim, S. A. and Cliver, D. O., 2010, Antimicrobial herb and spice compounds in food. *Food Control,* 21: 1199-1218.

Tandel, D. H., Sabalpara, A. N. and Pandya, J. R., 2010, Efficacy of Phytoextracts on Macrophomina Phaseolina (Tassi) Goid causing leaf blight of green gram. *International J. pharma and bioscience*, 2: 1-5.

Tapias, S. E., Gilpin, X., Ren, L., Wei, B. C., Fuchs, K. K., Tanabe, M., Lanuti, H. C. and Ott., 2015, Assessment of proliferation and cytotoxicity in a biomimetic 3-dimensional model of lung cancer, *Ann Thorac Surg,* 100(2): 414-421.

Tembo Y, Mkindi AG, Mkenda PA, Mpumi N, Mwanauta R, Stevenson PC, Ndakidemi PA, Belmain SR, 2018, Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming benefcial arthropods. *Front Plant Sci* 9:1425.

Thiba, K., Wai-Fong, Y. and Kok-Gan, C., 2012, Inhibition of Quorum Sensing-Controlled Virulence Factor Production in Pseudomonas aeruginosa PAO1 by Ayurveda Spice Clove (Syzygium Aromaticum) Bud Extract, *Sensors (Basel),* 12(4): 4016-4030.

Tian, J. Ban, B., Zeng, H., He, J., Bo, H. and Wang, Y., 2011, Chemical composition and antifungal activity of essential oil from Cicuta virosa L. var. latisecta Celak. *Int. J. Food Microbiol.,* 145: 464-470.

Tian, J., Ban, X. Q., Zeng, H., He, J. S., Huang, B. and Wang, Y. W., 2011, Chemical composition and antifungal activity of essential oil from Cicuta virosa L. var. latisecta Celak. *Int. J. of Food Microbiol.,* 145: 464-470.

Tongnuanchan, P. and Benjakul, S., 2014, Essential oils: Extraction, bioactivities, and their uses for food preservation. *J. Food Sci.*, 79: 1231-1249.

Verghese, S., Maria, C. F., Mullaseri, A. S., Asha, M., Padmaja, P. and Padhye, A. A., 2004, Aspergillus endocarditis presenting as femoral artery embolism. *Mycoses*, 47: 252-256.

Vincent, J. H. 1947. Distortion of fungi hyphae in the presence of certain inhibitors. *Nature*, 159: 850.

Williamson S, Ball A, Pretty J, 2008, Trends in pesticide use and drivers for safer pest management in four African countries. *Crop Prot* 27:1327–1334.

Wyckhuys KAG, Lu YH, Morales H, Vazquez LL, Legaspi JC, Eliopoulos PA, Hernandez LM, 2013, Current status and potential of conservation biological control for agriculture in the developing world. *Biol Control* 65:152–167.

Willem, F., Broekaert., Frankt, R. G., Terras., Bruno, P. A., Cammue, J. and Vanderleyden., 1989, An automated quantitative assay for fungal growth inhibition. *FEMS Microbiol. Letters*, 69: 55-59.

Wilson, J., O'Brien, T. and Orton, F. P., 2000, Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, *European J. of biochem.,* 267(17): 5421-5426.

Wolf, K. A., Daly, J. E., Reing, S. F. and Badylak., 2012, Biological scaffold composed of skeletal muscle extracellular matrix. *Biomaterials,* 33(10): 2916-2925.

Xu HX, Yang YJ, Lu YH, Zheng XS, Tian JC, Lai FX, Fu Q, Lu ZX, 2017, Sustainable management of rice insect pests by nonchemical-insecticide technologies in China. *Rice Sci* 24:61–72.

Zalata, N., Lammertijn, A., Christophe, F. H. and Comhaire., 1998, The correlates and alleged biochemical background of the resazurin reduction test in semen. *Int J Androl,* 21(5): 289-294.

Zhang, A., Jiang, M., Zhang, J., Tan, M. and Hu, X., 2006, Mitogen-Activated Protein Kinase Is Involved in Abscisic Acid-Induced Antioxidant Defense and Acts Downstream of Reactive Oxygen Species Production in Leaves of Maize Plants. *Plant Physiol*.,141: 475-487.

TERMS OF REFERENCE

FOR EVALUATION OF THE PROJECT ENTITLED "HIGH THROUGHPUT FUNCTIONAL CHARACTERIZATION OF INSECTICIDAL/NEMATICIDAL MOLECULES TO CONTROL INSECTS AND NEMATODES" IMPLEMENTED DURING THE PERIOD 2016-17 BY UNIVERSITY OF AGRICULTURAL SCIENCES, RAICHUR AT DEPARTMENT OF MOLECULAR BIOLOGY AND AGRICULTURAL BIOTECHNOLOGY. C.A. RAICHUR

1. **Title of the study :**
   Highthrouput functional characterization of Insecticidal/Nematicidal molecules to control Insects and Nematodes

2. **Department /Agency implementing the Scheme:**
   Dept. of Molecular Biology and Agricultural Biotechnology
   University of Agricultural Sciences, Raichur

3. **Project approval No. (Sector):** KA/RKVY-AGRE/2016/820
   Year of Start: 2016-17
   Year of Conclusion: 2018-19
   Total budget of the project: 2016-17 : 28 lakh
   2018-19 02 lakhs

4. Back ground and the context

Spodopteralitura (Fab.) is a polyphagous insect pest of national importance causing economic damage to 50 crops viz., tobacco, cole crops, castor, cotton, sunflower, chilli, groundnut, rice, tomato, tobacco, citrus, cocoa, potato, rubber, castor, millets, sorghum, maize estimated loss is 10 -12% world wide

Many crops grown as vegetables are susceptible to the nematode particularly tomato, okra, cucumber, melon, carrot, gourds, lettuce and peppers. Estimates of vegetable crop losses due Meloidogyne species, mainly *M. incognita* and M.javanica, have ranged from 17 to 20%

WHY SCREENING:

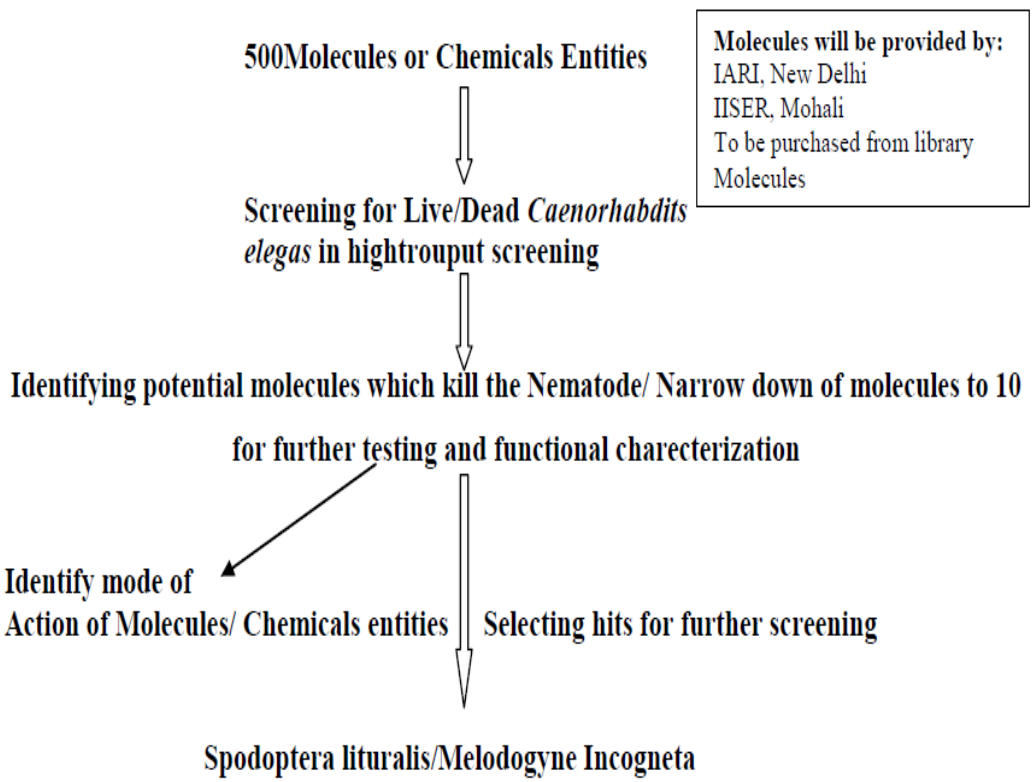
Hence obtaining chemical entities controlling these pests is a major challenge in this direction and public funded institutes are screening molecules one by one approach and this approach is going to consume long time for screening and laborious and investigator biases are involved. Hence, we are developing highthrouput screening

platform for chemical molecule screening/plant derived extract screening/ bacterial and fungal origin molecule screening by integration of imaging, software and automated motion detection programmes and analysis to hesten/ fasten the screening process and identify molecular targets of the hit molecules. Hence, the approach is novel.

NOVELTY INVOLVED:

1) Automated screening and data analysis
2) Screening first on model system: *Caenorhabditis elegans* based survival or death as endpoints for identification of hit molecule
3) Hit molecules will be tested on:
-*Spodopteralituralis*
-*Meladogyne incognetta*
4) Identifying molecular targets of hit molecules
5) Registration channel through UAS, Raichur for farmers utility

SCHEMATIC REPRESENTATION



5. The Objectives: The objectives of the project are as follows

1) Obtaining chemical scaffolds/ molecules for screening
2) Developing highthrouput screening platform
3) Testing the molecules for their effectiveness
4) Evaluation of efficacy on major insect/pests

6. Present status of the project:

The project was carried out in the Department of Molecular Biology and Agricultural Biotechnology, A.C. Raichur during 2016 -17 to 18 -19 with a funding of 30 lakhs and screened chemical scaffolds for nematicidal and fungicidal activity and found seven chemical compounds to be effectively controlling nematodes at 10ppm concentrations. Similarly screened 35 plant extracts and 15 essential oils for controlling *Aspergillus flavus, Fusarium oxysporum uduum* and *Rhizoctonia bataticola* control and two essential oil formulations found to be every effective. Further testing for the control of powdery mildew was tested on chilli and berr among tested essential oils two formulations found to be effective further testing in field conditions and large scale testing is in progress in the form of Demand driven projects supported by UAS, Raichur.

7. Out come of the project :

a) Developed highthrough put screening platform

b) Standardised *Caenorhabditis elegans* culturing, testing, evaluation and end points of the study

c) Screened molecules/plant extracts/ essential oils for the control of fungal pathogens/ nematodes

d) Two formulations to control fungal pathogen is under field testing.

e) Nematode control was also successfull by two chemical compounds at 50ppm and essential oil formulations.

f) Field testing for the powdery mildew control is in progress.

8. **Assets**: Include building, eqipments - all the assets purchased under the project.

Sl.No.	Name of the asset	Date of purchase	Qty. (Nos.)	Total cost (Rs.)	Purpose of purchase
1	Automated flourescence cell analyzer imaging system with controlled environment incubator	30/03/2017	01	19,99,999.00	Screening nematicidal and fungicidal activity
2	Trinocular stereozoom microscope	1/12/2016	01	97,325.00	Observing live death of nematodes and monitoring growth
3	Microscopy imaging camera	10/03/2017	01	86,000.00	Screening nematicidal and fungicidal activity and fungal growth monitoring
4	Camera bench regular	06/03/2017	01	40,000.00	Mounting the cameras for imaging studies
5	Small incubator	22/1/2018	01	10.000.00	Growth of fungus
6	Photo scanner for multiple plate scanning and image acquisition	19/10/2016		45,000.00	Hight throuput image scanning and evaluation by softwares for live/ death classification
7	UV Trans illuminator	17/03/2017	01	45,000.00	Fungal exudates evaluation

8	Camera stand inside glove box and smile drive	18/01/2018	01	25,000.00	For recording nematode movement for live death classification on exposure to chemical compounds

9. Where the project is undertaken: University of Agricultural Sciences, Raichur
Places to visit to evaluate the project: Department of Molecular Biology and AGRICULTURAL BIOTECHNOLOGY, College of Agriculture Raichur. UAS, Raichur

10. Evaluation Questions and minimum expectations:

a) How many number of chemicals / plant extracts and essential oils have been screened
b) Among screening the results obtained to control any disease or pests nematodes
c) Any formulations in progress
d) Is testing in field is in progress
e) Any commercialization possibilities
f) How to commercialize the product
g) Is registration needs to be carried out
h) Whether needs any further molecular testing

11. Evaluation Methodology and sampling: Evaluation can be done in two formats for this study
I) EVALUATION IN LABORATORY and II) Evaluation in field

I) EVALUATION IN LABORATORY

a) Invitro laboratory culturing of the fungal inoculants
b) Laboratory culturing of the nematodes both *C elegans* and root knot nematode
c) Preparing emulsifiers
d) Testing of the effective formulations with dose dependent
e) Recording of the results by microscopy/photographically
f) Analysing results

II) Evaluation in field

The formulations are tested for the control of powdery mildew of berr and chilli. This can be evaluated by spraying the specified dosage of formulations in the field with the incidence of powdery mildew seven days gap for three sprays is recommended. It will control the powdery mildew.

12. Deliverables:

After screening of various chemical entities/ plant extracts and essential oils. Few molecules showed to control nematodes at 50 ppm and also essential oil formulations. Further two formulations found to control powdery mildew and fungal diseases. These formulations are in various stages of testing under field conditions.

Two formulations are under testing in the field.

12. Duration and time schedule for the study: One month

13. Quality expected from the evaluation reports:

The formulations are in equivalent commercial product expectations. Hence quality is of commercial grade can be expected

14. Recommendations:

15. Cost and Schedule of budget:

16. Minimum qualification of the consultant: Ph.D

17. Providing Oversight

18. Contact persons:

Dr. Kisan .B. Asst. Prof and Head Department of Molecular Biology and Agricultural Biotechnology, A.C. Raichur 594101. Kisanb1@gmail.com

EVALUATION TEAM MEMBERS

Sl. No.	Name	Designation
1	Dr. B. C. Suryanarayana	Principal Investigator
2	Dr. M. A. Shankar	Associate Investigator
3	Sri. Siddaraju	Associate Investigator
4	Dr. A. R. V. Kumar	Subject Matter Specialist

Dr.Suryanarayana, B.C. is a doctorate in Agriculture with specialization in Agronomy and is a Certified Associate of Indian Institute of Banking (CAIIB), Fellow of Indian Institute of Valuers. He worked in State Bank of India from the year 1981 to 2014 as a Technical Officer and retired as Asst. General Manager (Rural Development). He is a practicing consultant in the field of Agriculture, Horticulture, poultry, dairy, fisheries and plant tissue culture and covered cultivation. He has about 35 years of experience in the field and has prepared several project reports for financial institution, written books in vanilla cultivation, anthurium, medicinal and aromatic crops, minor irrigation, poultry and dairy farming. He has appraised more than 6,000 proposals in agriculture and related fields for funding by the Bank and has also been involved in many studies relating to development of Agriculture and allied activities. He has served as a General Manager in a bio-fertilizer, bio-pesticides and organic manures manufacturing company and is also a Technical Director in a company involved in manufacture of agricultural implements and equipment.

Dr. M. A. Shankar is a doctorate in Agriculture with specialization in Agronomy. He is former Director of Research, University of Agricultural Sciences, Bengaluru and presently the Executive Member of Institution of Agricultural Technologists, Bengaluru and Co-Chairman of Agribusiness Consultancy Subcommittee. He has implemented 51 research projects for the University funded by International organizations, Central and State governments, Private firms. He has guided 6 Ph. D. students and 15 M. Sc., (Agri) students. As Dean of College of Agriculture, Hassan, he has, with his administrative skills, streamlined accounting, financial, academic and administrative issues. He has been involved in review and evaluation of Technical Reports of 32 All India Co-ordinated Research Projects (AICRP) spread all over India. He has also evaluated 11 operational research projects for the technical feasibility and implementation. He has published 173 peer reviewed research papers. He has also penned 54 booklets and books for the University. He has vast experience in evaluation studies of projects.

Sri. Siddaraju is a Graduate in Agriculture with more than 35 experience in the field of Agriculture. He has served in the Karnataka State Department of Agriculture (KSDA) as Asst. Agricultural Officer in Farmers' Training and Education Centre, Soil Testing laboratory and as Subject Matter Specialist. He was Deputy Director of Agriculture (Commercial Crops) for 6 years, District Watershed Development Officer for 2 years. He has also been Joint Director of Agriculture (Inputs) for 5 years. He was involved in preparation of Annual Programme Planning booklets pertaining to Agricultural Inputs in Department of Agriculture. After retirement, he is serving as Chairman, Agriculture Consultancy Subcommittee, Institution of Agricultural Technologists, Bengaluru and has been actively involved in evaluation studies of projects.

Dr. A.R.V. Kumar, is a doctorate in Agriculture with specialization in Agricultural Entomolgy from University of Agricultural Sciences, Bangalore. He served in the University in various capacities and retired as University Head of Entomology Department, Professor of Entomology. He has worked on aspects of Pest management in different cropping systems and for an extended period on the management of White grubs in different cropping systems. He has built up a collection of over 50,000 white grub specimens of India at the department. He has also worked on the use of neem in pest management and Insect Tolerant Transgenic Crops. He has guided both masters and Ph.D. students on various aspects of Pest Management and Insect Taxonomy. He has taught Insect Morphology, Principles of IPM, Insect Ecology and Insect Taxonomy at the University. He took special interest in the development of infrastructure at the Department of Entomology, set up a molecular biology lab and to set up the First Butterfly Park at the Banneraghatta National Park, Bengaluru. He has 95 research articles and Two edited books to his credit. Two of his publications are being extensively used in teaching Community Ecology. Currently he is working on the development of mass multiplication techniques for several insects as sources of animal protein.