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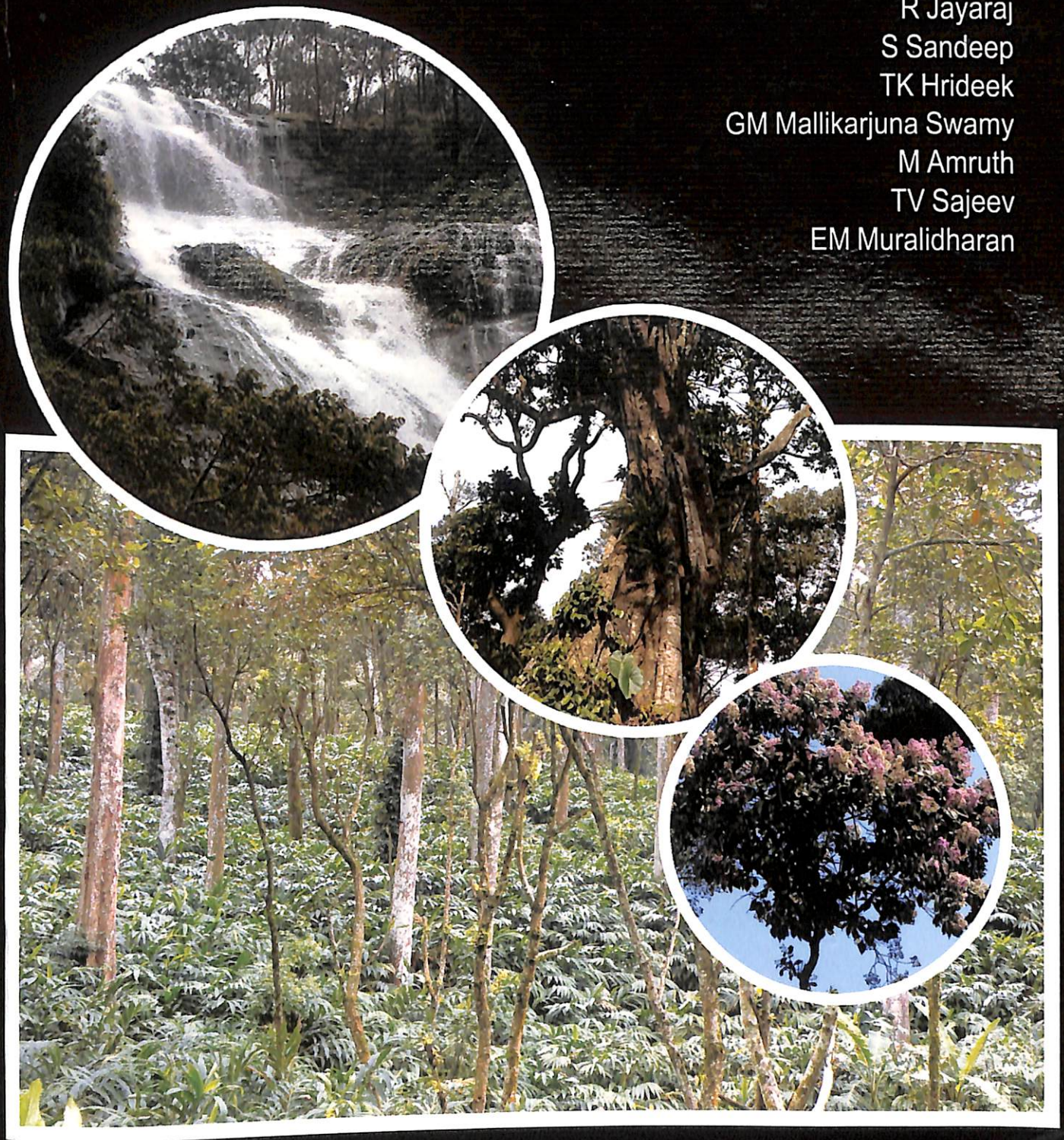

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Peechi-680 653, Thrissur

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Final report KFRI RP 632/2011

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PROJECT DETAILS

Title of the Project	:	**KFRI RP – 632/2011** - Environmental impact of pesticide application in Cardamom Hill Reserves (CHR) of Southern Western Ghats
Objectives	:	1. Identification of pesticide affected regions in CHR and adjacent forests 2. Study of pesticide residues and its biological implications in the terrestrial environment 3. Role of biogeochemistry in the transfer, distribution and degradation of pesticides
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Period	:	3 Year (01 January 2012 – 31 December 2014)

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INVESTIGATORS

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ABSTRACT

Kerala has a unique landscape where agricultural fields lie close to forest lands, consequently most forest ecosystems are disturbed in numerous ways due to widespread anthropogenic activities, mainly exhaustive agricultural interventions. Cardamom Hill Reserves (CHR) of southern Western Ghats is one such example. In CHR cardamom grows naturally since centuries and presently this region is intensively managed for cardamom cultivation. Due to the high incidence of pests and diseases on this high value crop, several insecticides and plant protection chemicals including toxic and hazardous pesticides were used to protect the crop from the pests for increasing the yield. However, the high use of chemicals have a negative impact on the ecosystem. Keeping these in view, the present investigation was undertaken to throw light on the negative impacts of pesticide application on the ecosystem health in the region.

Major crops in the study area were cardamom (78.13 %), mixed crops (9.38 %), tea/coffee (1.04 %) and banana/cocoa (2.00 %). The survey conducted in the area indicated the extent of use of pesticides among cardamom plantations as: organophosphorus (45.6%), neonicotinoids (12.66 %), pyrethroids (12.66 %), unknown/not declared (18.99 %), carbamates (5.06 %), GABA receptor blockers (2.53 %) and organochlorides (2.53 %).

The soil-stress studies using *Eisenia fetida* proved the avoidance of unfavorable environment by soil invertebrates leading to aggressive movement of earthworms from the pesticide contaminated region. *In situ* toxicity studies established the toxic effects of chlorpyrifos on *Eisenia fetida* evidenced by high mortality rate, weight loss, increased lipid peroxidation levels, decreased total protein levels and glutathione depletion. Soil microbial dehydrogenase assay revealed the low microbial activity in the soils of the study region. Photosynthetic efficiency test and karyotype analysis conducted on dominant plants of the region identified the stress level of common plant species in CHR under continuous pesticide application. Ball metaphase noted in *Artocarpus heterophyllus* suggests direct destructive effects of pesticides at chromosome level. *Aspergillus niger, Blastomyces dermites, Cladosporium herbarum, Collectotricum gloesporioides, Paecilomyces sp.,* and *Penicillium crysogenum* were identified as promising pesticide degrading microbes in the study area and they are promising candidates for developing novel microbial consortia for pesticide degradation in soils.

The chemical characterization of the soil revealed that the soil pH in CHR vary between 3.56 - 6.98 and nearly 65 % of the samples were very strongly acidic to extremely acidic. Soil organic carbon in the soils were very high in most of the soils, but the restricted decomposition at low temperatures leads to wider variation in C:N ratio in these soils. The soils were adequate in P and K. The pesticide adsorption studies revealed that the soils of CHR have good retention capacity for the pesticides chlorpyrifos, quinalphos and dimethoate. The adsorption isotherms also show that, though the amount of maximum pesticide adsorbed decreases with pH, the binding strength increases. Column-leaching experiments show that the retained pesticides are highly vulnerable to leaching under continuous rains which can easily pollute ground water and nearby water bodies.

The field experiments at CRS Pampadumpara show that under high doses of pesticide application, there is a gradual accumulation of all the pesticides in soils and plant bodies. In soils this will either be degraded or leached and in plants the decomposition is very slow as accumulated levels were seldom found to decrease in any of the experiments during the analyzed period. The plant-accumulated pesticides have a high potential for bioaccumulation as it moves along the food chain. Hence it is always advisable to adopt methods of pest control with no or minimum levels of chemical pesticides. Over all the study revealed the degraded nature of the ecosystem in the region with adverse effects on the soil biota and plants due to unscientific pesticide use.

Introduction

Introduction

In Kerala's landscape, forest land and agricultural fields are in close proximity across the state. These forest ecosystems are disturbed in many parts due to extensive anthropogenic activities, mainly intensive agricultural interventions. High usage of fertilizers and pesticides in these farm lands are always creating disturbances in forest ecosystems and wildlife around. Major rivers in Kerala arise as streams in the high ranges of Western Ghats and pass through forest land as well as big plantation regions. One such major example is Periyar River, bordering Cardamom Hill Reserve (CHR), and adjoining the Periyar Tiger Reserve, reserve forests of the Ranni, Konni and Achankovil Forest Divisions, the Srivilliputtur Wildlife Sanctuary and reserved forests of the Tirunelveli Forest Divisions. CHR is a naturally cardamom growing area since centuries and presently one of the highly managed regions for cardamom cultivation. CHR has a peculiar microclimatic condition where the growth of cardamom is augmented naturally with lots of shade trees, high canopy cover and high humid condition where cardamom plants grow as under growth. CHR once had lots of wet lands and thick forest on the slopes, which helped meter the release of water into the streams and rivers.

Cardamom (*Elettaria cardamomum* Maton.) universally called "Queen of spices" is one of the costliest spice after saffron (*Crocus sativus* L.) and vanilla (*Vanilla planifolia, V. fragrans* or *V. vanilla* Jacks.). Cardamom belongs to the family "Zingiberaceae" and has the C4 photosynthetic pathway (Raghavendra and Das, 1976). Cardamom was expected to be very sensitive to changing climatic conditions and used to be grown as a shade loving crop under thick evergreen forest canopy. But in the recent changing climate conditions, cardamom performs like summer annuals bearing temperature to a significant level. Considering the high economic value of cardamom and the frequency of pathogen and insect spell on the crop, many insecticides and plant protection chemicals including toxic and hazardous pesticides have been used to protect the crop from the pests and to increase the yield.

Since intensive agricultural practices are followed in the CHR, high use of pesticides are reported from the region. Of late, most of the world's cardamom cultivation includes high input of chemical pesticides (12-15 rounds of pesticide application per season) and fertilizers (Miniraj and Murugan, 2000; Murugan et al., 2006). A recent report confirms the high usage of pesticides in cardamom cultivation in the CHR region with 650 tonnes of pesticide active ingredients applied during 2009 alone. As per the cultivation practices in the region, cardamom needs 15-18 rounds of

pesticide sprays per year. Continuous cardamom cultivation has led to soil degradation and disturbances in the ecosystem with increased acidity, accumulation of heavy and rare metals (Cu, Pb and Zn) and pesticide residues (Endosulfan, Chlorpyrifos, DDT), leaching of N and P through run off and surface waters (Murugan et al., 2011a,b).

Recent reports reveal that 60 % of the total pesticides used in Kerala have been applied on cardamom and other crops in Cardamom Hills. The use of pesticides in agroecosystems offers high yield. Even though much data and literature on pesticide usage on sustainability of cardamom ecosystem are available, precise information is lacking and published literature is very limited. Pattern of use of pesticides primarily depends on types of pest attack and crops and varieties. Further climate, season, soil reaction and fertility also plays crucial role in the selection of appropriate pesticides. These are the main factors considered to affect the pest population dynamics of crops.

Quantifying the effects of agricultural management practices on ecosystem functions is essential to maintain the sustainability of environment. It is universally accepted that, pesticide use in the agricultural systems eventually enters the food chain and increases risks to human health, the natural environment and also has a social impact also. However, effect of pesticide use in crop management are habitually measured by the yield increase gained. While sustainability of agriculture is evaluated, income generated versus the input cost such as seed, fertilizers, pesticides prices and labor coast is generally taken to consideration, neglecting the environmental impact of agrochemicals in the long term.

It is understandable that increase in pollution level in agricultural fields and environment increases the human, natural and social costs which could reduce the livelihood security and quality of life of primary beneficiaries such as farmers and farm workers. This is usually justified in terms of yield benefits versus input costs. In such a profit calculation, human health hazards, ecosystem and environmental damage are often neglected. Therefore, establishing a crop as profit making, based on the economic yield alone is not justified since it is not obvious so. Appreciating the paybacks of pesticide use merely on the basis of economic yield may result in a surge of their use in agriculture management and subsequently leads to ill effects from such an unjustifiable use. The economic analysis based only on conventional costs may claim that the high agrochemical inputs may be beneficial. This is based

on the belief that technological advancements have only positive effects to human society (Meghani, 2008) and such developments will work beneficial with increasing population growth, poverty, hunger and malnutrition (FAO, 2004). However such an evaluation does not take into consideration of environmental impacts like pollution of natural resources including contaminated food materials, ecosystem disturbances and long-term negative impacts on the human health such as health problems, cancer and genetic abnormalities. Similarly, it is apparent that conversion of agriculture to highly rigorous production oriented system can bring out ecological and environmental disturbances in addition to socioeconomic and health related problems in CHR.

The forest areas in CHR are a part of southern Western Ghats classified as tropical evergreen forests (tropical rain forest). The southern Western Ghats is one of the important biodiversity hot spots of the world (Myers, 1988). The optimal climatic and environmental conditions that prevail in this region resulted in high species richness and high number of endemic species (1500 species, 56%) and high biodiversity (Pascal, 1988; Ramesh et al., 1991). Even though cardamom is considered to be an undergrowth, presently major varieties of cardamom require less shade compared to earlier varieties. Owing to this reason, farmers reduce the canopy cover in the CHR areas. This is practice has led to a considerable loss in biodiversity of the region.

Another factor which needs attention is the changing weather conditions in the region. Current global warming along with extreme anthropogenic activities and degradation of forests can upset the micro and macro climate of CHR. Even though the Periyar river is associated with CHR very closely, the effect of pesticides on the terrestrial and aquatic organisms, soil make up and micro-environment of that region has never been explored. Pesticides are designed with a concept that pest is an unwanted organism in an unwanted place, hence needs to be eliminated. In order to find out ways and means to kill the pest we might be using pesticides of different chemical nature, which might be damaging to humans as well as other living organisms and plants. Pesticides used for agricultural or domestic purposes are released into the environment and cause unintended effects on the ecosystem.

The average Indian consumption of pesticide is far lower than many other developed economies, but the problem of pesticide residues is very high in India. A vast majority of the population in India is engaged in agriculture and is therefore

exposed to the pesticides used in agriculture. Even though high pesticide use has been established in CHR region by various researchers (Murugan et al., 2011; Vinoth Kumar et al., 2009), the effect on the microenvironment has been ignored. Keeping all these in view, the present investigation is to determine the status of pesticide residues and its implications on the ecosystem health and function in the region. The present study involves the assessment of land degradation in terms of physio-chemical characteristics of the soil, pesticide persistence and toxicity in different matrices of the soil, pesticide effect on forest trees and microflora. Through the present work we have tried to assess the pesticide movement, leaching and adsorption based on the local soil parameters

By keeping in view of the above, the objectives of the present study are ;

1. Identification of pesticide affected regions in CHR and adjacent forests

2. Study of pesticide residues and its biological implications in the terrestrial environment

3. Role of biogeochemistry in the transfer, distribution and degradation of pesticides

Review of Literature

Pesticide use pattern – Indian agricultural systems

Chemical pesticides have contributed immensely to the increased agricultural production, control of disease causing insect and pests in farming sector both in plantation sector and animal farming and also in human health sector (Bhatnagar, 2001). The demand for increased food production to cater the growing population has led to adoption of high yielding varieties and effective pest management strategies (Agoramoorthy, 2008; Lakshmi, 1993). Worldwide, pesticide use causes 3 million cases of poisoning and 220 thousand deaths and about 750 thousand instances of chronic illnesses every year (WHO, 2005).

In tropical countries like India, the high humidity and temperature favors the rapid multiplication of pests leading to increased crop loss (Kannan et al., 1992). Annual crop loss in India due to pests amounts to Rs.6000 crores, of which 33 % is due to weeds, 26 % by diseases, 20% by insects, 12%by birds and rodents and 11 % by others (Rajendran, 2003). The magnitude of this problem tend to increase with appearance of more pests and more target crops in the years to come. Use of DDT for malaria control and BHC for locust control in 1948-49 were the initial cases of use of synthetic pesticides (Gupta, 2004). A BHC plant established in 1952 near Kolkatta was the first Indian pesticide production unit, after which two DDT manufacturing units were set up by Hindustan Insecticides Limited. This was followed by Union Carbide India Limited plant at Bhopal in 1969 for pesticide formulations. Many formulations such as, Sevin - carboryl insecticide, Temicard and Aldicarb were synthesized and sold in India from this plant till its closure in 1989 after Bhopal MIC disaster. Presently, Indian pesticide industry has more than 500 pesticide formulations by large and medium scale producers with in the country (Abhilash and Singh, 2009). Major share of this formulations goes to dusting powder (85%), followed by water soluble dispensable powders (12%) and emulsification concentrates (2%).

In India, the annual pesticide business is estimated to be Rs.5000 cores and is expected to grow faster in coming years. This is directly proportional to agricultural production requirements in order to meet the requirements of growing population. The statistics of previous years shows a substantial increase in the pesticide use in Indian agriculture (Table-1)

Among the pesticides used in India, some of them are extremely toxic. Toxic chlorinated pesticides such as p,p'- dichlorodiphenyl trichloroethane (DDT), hexa chlorocyclo hexane (HCH) and pentachlorophenol (PCP) are still manufactured and used in the country.

India is the producer of 90000 metric tons of pesticides, which is the highest in Asia and 12th in the world. Worldwide consumption of pesticides is about 2 million tons per year, with USA-24 %, Europe - 45 % and rest of world - 25 %. In comparison to other Asian countries like Japan (12.0 kg/ha) and Korea (6.6 kg/ha), the use in India

Table-1 : Consumption of Pesticides (Technical Grade) in India

Years	Consumption (in ' 000 Tons)	Years	Consumption (in ' 000 Tons)
1950-51	2.35	1994-95	61.36
1960-61	8.62	1995-96	61.26
1970-71	24.32	1996-97	56.11
1971-72	29.54	1997-98	52.24
1972-73	35.16	1998-99	49.16
1973-74	50.43	1999-00	46.20
1980-81	45.00	2000-01	43.58
1981-82	47.00	2001-02	47.02
1982-83	50.00	2002-03	48.30
1983-84	55.00	2003-04	41.00
1984-85	56.00	2004-05	40.67
1985-86	52.00	2005-06	39.77
1986-87	50.00	2006-07	41.51
1987-88	66.90	2007-08	44.77
1988-89	75.89	2008-09	43.86
1989-90	72.00	2009-10	41.82
1990-91	75.00	2010-11	55.54
1991-92	72.13	2011-12	52.98
1992-93	70.79	2012-13	46.00
1993-94	63.65	2013-14	60.00

(0.5 kg/ha) is much lower. Among the different classes of pesticides used, organochlorides (40%) are highly used followed by organophosphates (Gupta 2004). However due to increase in awareness on the toxicity and persistence, organophospate-based pesticides have overtaken the organochlorides in the last decade.

The use of pesticides were for six major sectors in India, (1) agriculture – for control of pests, weeds and rodents, (2) public health – for control of vector borne diseases like malaria, Japanese encephalitis, dengue etc (3) industries – weedicides and fungicides (4) domestic – weedicides, control of garden pests and insects (5) personal – application in clothing and skin care and (6) construction materials – incorporation in paints, coatings, sheets etc.

Yearly agricultural pattern and climate also has a greater role to play in pest infestation and application of pesticides. Modernization has led to the change in external inputs during the process. Chemical input mainly fertilizers and pesticides have become an integral part of high yield farming. With change in cropping seasons and climate conditions, there has been a change in target plants and infestation by pests. For example, areas with coarse cereals, small millets and barley were decreasing and continuous high yielding crops like cotton, paddy etc are in rise. There are number of factors which affects safety of persons handling pesticides during storage and application. This is not only a problem in India, but in many developing nations of the third world. The major issues are lack of training in handling and use, ignorance about potential dangers, lack of knowledge on labeling and toxicity, intake of food materials during spraying or application, lack of proper safety equipments and reuse of containers among others.

Pesticide use in Kerala

Pest management of agricultural crops has always been a troublesome issue in farming sector. Mostly productivity is inversely proportional to the pest infestation and with the present scenario of changing climate, the crops become increasingly vulnerable to more and more pest infestations. There has always been a changing trend in the use of pesticides, mainly based on the cropping patterns and infestation levels. Pesticide usage (technical grade insecticides, fungicides, weedicides and rodenticides) in Kerala over last two decades (1991-2008) has been 13526.37 metric tons (MT). Of this 1381.3 MT was in 1994-95, followed by 1328.10 MT in 2001-02 and the lowest 271.96 MT in 2003-04. (Indira Devi, 2010). Among this, fungicides

dominate the pesticide market in Kerala (73 %) followed by insecticides (20 %). However this is contrary to the global and national pattern of continuous increase in use.

Indira Devi (2010) has done a complete review of the pesticide use in Kerala over a decade and brought out some interesting findings. Though there has been a decline in the use, the study found that the use of highly toxic pesticides were on the rise. In most of the cases the applications are of prophylactic in nature. The risk perception analysis on pest incidence and agricultural loss has naturally increased the pesticide use, despite the fact that chances of such pest attacks are dismally low (Indira Devi, 2007). In commercial agriculture, chemical plant protection methods are adopted on a preventive basis with application of pesticides right from the planting or transplanting of the seedlings. However most of the recommendations of agencies like Agricultural Department, Kerala Agricultural University (KAU) and commodity boards are to use the pesticides in a need based manner (Indira Devi, 2009). The Government of Kerala has banned the sale and use of endosulphan, after many reports over environmental and human health issues due to the aerial spraying in cashew plantations of Northern Kerala. Despite the ban, its use in pineapple and mango farms across state has been reported through informal discussions (Indira Devi, 2010).

Recommendations on dose, application methods and timings for a particular pest or crop is a grey area which was never addressed properly. Even though the recommendations from Agricultural Department, KAU and commodity boards are in place, however many times farmers procure the chemicals based on dealers or fellow farmer's advice. Farmers usually opt for quick results and apply most toxic chemicals, even when the safer ones are available. More over the agents and representatives from manufacturer's or dealers approach the farmers for providing technical advice on pest infestation and sell the chemicals. This is a highly undesirable practice which may lead to unintended mishandling of highly toxic chemicals.

The application modes of these chemicals also have a great role to play in their potency and toxic effects. Drenching in the case of systemic agents and spray application in the case of contact agents are major routes of application. Scientific handling of pesticides is another aspect to be considered. In handling of most of the pesticides, recommendations are for using protective cloths, face masks, goggles,

head cover, gloves and boots. However, these recommendations are ignored in common practice. In Kerala, most of the protective measurements are not taken in to consideration during pesticide applications as recorded from the paddy farm workers in Kuttanad (Indira Devi, 2010). Among the farm workers in Kerala, the awareness on toxicity, ecological impacts and human health impacts were varies at different levels. Only less than 3 % follow instructions, Even though the toxicity data is represented with colour code in bottle, 99.5 % of the common farm workers do not understand the toxicity implications. There is a need for farmers to be trained in the proper use of these chemicals and to understand the meaning of codes and labels.

Cardamom cultivation is highly dependent on pesticide and fertilizer applications. The high economic value of cardamom encourages the farmers to opt for intensive agricultural practices with chemical aids. However sustainability of any such crop can be ensured only by the understanding the effects of these management practices on the ecosystem. Other than the ecological impacts, adverse human health effects are also reported due to high pesticide use. Extension of cardamom agricultural practicing areas in CHR is unlikely to increase due to the specific microclimatic requirements of the crop. Therefore, intensification of agricultural practices are the way forward to increase the productivity of this crop and that has been practiced by the farmers of the region for many years.

Pesticide usage for the sustainability of cardamom cultivation in CHR should always be assessed in terms of ecological significance of the region. There are two major aspects needed to be considered during the assessment are i). history, ecological niche and biodiversity importance of the region and ii). nature, rate and fate of pesticides in the microclimatic conditions of agro-ecosystems. All the available information's in both of these areas are put together to analyze the present state of affairs.

I) History, ecological niche and biodiversity importance of Cardamom Hill Reserves

Historical Importance

Cardamom Hill Reserves consists of evergreen forests which are richest in terms of plant biodiversity within the Western Ghats (Pascal, 1988). The humid forest of this area stated with the highest extent of endemism in Western Ghats (Ramesh and Pascal, 1977). The most favorable and optimum climatic and environmental conditions that prevail in southern Western Ghats is the reason for its species richness and endemism leading to the presence of highest number of endemic species (1500 species) (56%) and highest biodiversity (Pascal, 1988; Ramesh et al., 1991). An area of 3880.5 sq km is brought under protection and conservation out of 22685.9 sq km in southern Western Ghats forests.

This region that falls in the Idukki district of Kerala is known for the wild growth of small cardamom (*Elettaria cardamomum*) as an undergrowth. This place has been identified to be one of the best areas for cardamom cultivation since centuries, hence named as Cardamom Hill Reserves (CHR). The CHR forest, according to official declaration made in 1897 spans across hills with altitude between 600 and 1200 m above mean sea level and as of today falls in the Devikulam, Udumbenchola and Peerumedu taluks. As noted in the historical records, CHR region was 344 sq miles (87,335 ha), however over time the area has shrunk. CHR is identified for the finest agroecology for cardamom farming with evergreen forest cover and well dispersed high rain fall.

History of cardamom hills starts in early 1800's when many cardamom gardens were established throughout the High Ranges (HR) adjacent to Bodinayakanur and Cumbum towns. Harvest of cardamom from wild was a source of income to the erstwhile kingdom of Travancore. A royal proclamation was made in 1897 for continuous increase of cardamom production and protection of the region along with biodiversity conservation. Conservation of forest cover was important for sustained cultivation and yield of cardamom. Further in 1935, Travancore government formulated rules for leasing out the CHR area for cardamom cultivation. The concern over the conservation of flora and soil led to such a legislation and based on this CHR was assigned to private persons including tribal peoples on registry and on lease for cardamom cultivation. The direction was for only cardamom cultivation in leased areas under CHR legislation maintaining the tree canopy cover preserving the microclimatic conditions, failing which the land

would revert to the government. The early planters realized the significance of the tropical wet evergreen forest ecosystem and the microclimate as the primary requirement of the crops and they were doing the farming without any harm to forest and forest ecosystem. The co-existence of cardamom and rain forests are still treasured. The rules restricted the commercial exploitation of timber from cardamom plantations. Cardamom become an important wild produce of Southern Western Ghats and over time it become a spice of high commodity value.

Rules and regulations for assessment of cardamom gardens and allotting lands for cultivation in cardamom hill area and Periyar Tiger Reserve were permitted by the Travancore Royal Dynasty H.H. Maharaja on 12th August 1905 in accordance with the concessions granted to Cardamom cultivators. A Superintendent of cardamom hills was appointed for the better administration of the area. Consequently, in 1910 this administrative structure was abolished and absorbed to Land Revenue Department with a sole authority of Superintendent and Magistrate of Cardamom Hills. The allotment of forest land on registry for cardamom cultivation was superseded in 1942 and the system of lease by auction was introduced. But in some cases where lands already given under Cardamom Rules 1935, were continued. The Forest Department was vested with powers for permission of felling trees, as a safe guard against indiscriminate felling. Consequently in 1944, some modifications in the rules regulating these leases came to effect. As per this, the cardamom hill lands already occupied and improved upon should be leased out to occupants without auctions with a lease period of 12 years.

Further to this in 1950, the control of the entire Cardamom Hill Reserves, were vested with Revenue Department and Forest Department had control only over the tree growth through government order # GO (MS) 2329/50/R dated 26/08/1950. However in 1952, the dual control system was abolished and the total control of the area was entrusted with Forest Department. This was further reviewed in 1958 and the duel control system was reintroduced. Consequently, "Rules for the lease of Govt. land for cardamom cultivation" was introduced in 1961 and in 1963, Revenue Divisional Officer, Devikulam was entrusted to look after the cardamom leases in CHR. In 1965, a Special Settlement Officer and Assistant Director of Survey was appointed to assist him. Many land assignment documents were issued since 1965 by Revenue Department and it is not clear that whether all those lands can now be considered as part of CHR land or not.

As part of the "Grow More Food" programme introduced after the Second World War, large extent of CHR areas were allotted to people for food crop cultivation later turned to agricultural crops like pepper, coffee, coconut ginger etc. This lead to the development of townships like Nedukandam, Kattapana, Kallar etc. During 1980's the scenario was changed to forest conservation. Implementation of Kerala Preservation of Trees Act 1986 Section-5, prohibited the cutting, uprooting, burning or otherwise destroying trees in Cardamom Hill Reserves areas except on the reasons that, the tree constitutes a danger to life or property or the tree is dead diseased or wind fallen.

Ecological importance and biodiversity

The Cardamom Hill Reserves situated in very a important region which is directly connected to many riverine systems in Kerala, major being Periyar river and its tributaries. It is connected to Palani hills and Periyar Tiger Reserves and thus forms a natural corridor for the passage of wildlife between these regions. Cardamom Hill Reserve, even after having undergone various types of land use still has a substantial amount of plant diversity. The initial planters and the fresh immigrants from the plains abandoned certain parts of the CHR due to its inaccessibility or due to failure of cardamom cultivation in early decades of 20th century. Rich diversity of plants occurs in those areas with the presence of many rare and threatened species. The administration of these areas are under a dual control system with the involvement of both Kerala Revenue and Forest Departments and poses confusion and uncertainty on the conservation of the species diversity. The presence of such a large number of species with a high percentage of endemism in CHR provides evidence of the immense value of the original vegetation of the CHR. The richness is comparable to any Protected Areas in Kerala. Cardamom Hills is surrounded by a number of Protected Areas in the entire stretch of the Western Ghats. Eravikulam National Park, the cluster of Shola National Park and Chinnar Wildlife Sanctuary in Kerala and the Anamalai Wildlife Sanctuary in Tamil Nadu are along the northern fringe of the Cardamom Hills. The Thattekadu and Idukki Wildlife Sanctuaries are to the west and the Periyar Tiger Reserve is along the southern border

The CHR is unique in its floristic and faunistic composition. About 40% of the 1040 plant species recorded from the area are endemic to Western Ghats. Thirty eight species of plants are critically endangered. Out of the 40 species of orchids recorded, 20 are endemic. *Taeniophyllun scaberulum,* an orchid, which was considered extinct

till recently has been rediscovered from the Reserve. The faunal diversity of CHR includes Nilgiri langur, leopard cat, barking deer, Malabar spiny dormouse and mouse deer. Fourteen of the 16 endemic birds of Western Ghats are seen in the area. A number of Uropeltids, the burrowing snakes are reported from CHR and the adjacent areas. The northern part of the CHR is an elephant corridor.

A study by Jha et al., (2000) demonstrates that the forest damage in the southern Western Ghats during the last quarter of the 20th century was about 25.6%. They ascribed this loss to the conversion of dense forests to plantations. This observation has more significance to Idukki district where plantation areas increased by 5.62 %. Cardamom is a spice which needs a substantial amount of shade and moisture. The decrease of the canopy cover by thinning may give more yields for a short period but in the long run it may make the soil unsuitable for any crop including cardamom. The inappropriateness of some of the areas of the High Ranges for cardamom cultivation, the dynamic nature of the price of cardamom in the market coupled with the high price for other cash crops, and/or the attitude of the new immigrants from the plains might have been the reasons for the large-scale conversions of cardamom to other crops. It is the canopy cover and flora of the CHR, which determines the survival of the sustainable cardamom cultivation in the High Ranges. Hence any devastation, will certainly be detrimental to the future of Cardamom Hill Reserves.

II) Nature, rate of use and fate of pesticides in the microclimatic conditions of agro-ecosystems.

Pesticides are a group of chemicals used for the destruction of insects, weeds, fungi, bacteria etc. They are generally called insecticides, fungicides, bactericides, herbicides or rodenticides. Most of the pesticides have the ability to destroy wide variety of pest or weeds, but some are developed against specific pests or pathogens. Most of these chemicals are designed in such a way to disturb the physiological activities of the target organism which leads to dysfunction and reduces its vitality. Pesticide residues may constitute a significant source of contamination of environment such as air, water and soil. This phenomenon could become a continuous threat to the co-existence of plant and animal communities of the ecosystem. Problems caused by pests lead to loss of about one third of the world's agricultural production every year even though the pesticide consumption is more than two million tons. In India, the loss amounts to more than Rs. 6,000 crores annually, by contributing factors such as weeds (33%), diseases (26%), insects (20%),

birds (10%), rodents and others (11%). Every year the magnitude of the problem increases by the appearance of newer pests and diseases (Rajendran, 2003).

The greater use of pesticides for high agricultural production has led to increased pollution of environmental compartments- soil, water and air. The characteristics of pesticides such as high lipophilicity, bioaccumulation, long half-life and potential of long range transport increases the chances of contaminating the air, water and soil, constantly even after many years of application. A study by Pimentel (1995) showed that only a small percentage (0.3%) of the applied pesticide goes into the target pest, the remaining 99.7% going somewhere else in the environment. Application of wide variety of pesticides have been advised to increase the crop productivity in tropical countries, because crop loss is severe due to high temperature and humidity, which are conductive to rapid multiplication of pests (Kannan et al., 1993; Lakshmi, 1993). As per a World Health Organisation study, 80% of all pesticides are used by developing countries . Due to lack of proper legislations, improper market regulations and ignorance shown by people, agricultural workers from developing countries are exposed to high levels of agricultural chemicals including pesticides (Smith and Jong, 2001). Among agriculturalists of developing countries, pesticide exposure is a primary occupational hazard (Wasseling et al., 2001; Konradsen et al., 2003; Coronado et al., 2004) which leads to health issues and environmental contamination associated with pesticide use (Mancini, 2005; Remor et al., 2009). Although farmers are considered to be the main risk group, formulators, loaders, mixers, production workers and agricultural farm workers are all extremely susceptible groups. The non-occupational hazards may be due to pollution of ecosystem or habitat as a whole such as from water, air and food. An estimate shows that deaths and chronic diseases due to pesticide poisoning numbers about one million per year worldwide (Environews Forum, 1999).
The overuse or misuse of pesticides is contributing adversely to the environmental health as well as the ecosystem services. Pesticides are reported to affect many aquatic and terrestrial species. Life in aquatic ecosystems such as microorganisms, invertebrates, plants and fish are badly affected by pesticides (Liess et al., 2005; Grande et al., 1994; De Lorenzo et al., 2001; Castillo et al., 2006; Frankart et al; 2003). In the Indian situation, massive use of pesticides have started since 1960's, when the "Green Revolution" was initiated. To maximize the food production, high levels of agrochemicals were used during those periods.

More than 700 pesticides are registered for use in the world (Tomlin and Clive, 1994), and many more continue to persist in the environment, even though they are no longer in use. The premature release of medicinal, industrial and agricultural chemicals has caused numerous environmental problems at all levels of life (APHA, 1980; Ecobichon, 1986). For the protection of human health and the environment, pesticide residues are routinely monitored in food, water, soil, and tissue samples. "Acceptable" residue limits have been set for various foods and environmental samples by regulatory agencies. A number of methods have been developed to detect the presence of pesticides in food (McMahon *et al.*, 1994; Fillion *et al.*, 1995) and the environment (Stan, 1995; Wagner *et al.*, 1994) to reduce the risks associated with pesticide exposure. There are various reports that describe sample handling, extraction and clean-up procedures in the determination and evaluation of pesticides and interpretation of results. The individual steps of the analytical procedure are designed according to the chemical structure of the analyte compounds and according to the character of the matrix. Chromatographic methods, in particular gas chromatography (GC) coupled with mass spectrometer and high performance liquid chromatography (HPLC), are the methods of choice for qualitative and quantitative evaluation of pesticides. Viden *et al.*, (1987) determined residues of triazines in forage and milk, the identity of the residues being confirmed by GC-mass spectrometry. Herbicides based on urea can be determined by HPLC. After thorough clean-up of the extracts, determination limits in the range 0.015-0.02 mg kg^{-1} could be achieved for plant materials using UV detection (Bolzoni and Dagnino, 1985; Goewie and Hogendoom 1985; Miliadis *et al.*, 1990) A review by Jozef and Jana (1993) gives a comprehensive report on various analytical methods like, GC, GC-MS, HPLC etc used for the detection of main structural groups of pesticides, i.e., triazines, phenyl- and sulphonylureas, carbamates, uracils and phenoxyalkanoic and arylphenoxypropanoic acids, and important degradation products (dealkylated triazines, substituted anilines and chlorophenols).

Several reports have indicated the toxic effects of pesticides on plants mainly on seed germination and cytological abnormalities. Possible mechanisms of the toxic action of pesticides during the germination of seeds have been discussed with emphasis on biochemical, histological, and cytological alterations. Some of the pesticides are reported to be beneficial for plant growth if used at lower concentration but becomes phytotoxic at their higher dose and bring about changes in the activity of some useful soil microorganisms (Tu, 1994; Wang Zhenzhong et al., 2002). The negative role of pesticide treatment on fertility and cytological

abnormalities was observed first in seed set of tobacco plants after the plants had been fumigated with nicotine sulfate (Kostoff, 1931). Plants can be affected directly by pollutants as well as indirectly through the contamination of soil and water. The responses of the plants are directly proportional to the severity these pollutants of the environment in which they are surviving (Levitt, 1941). The major adverse effect of organochlorine (OC) pesticides in animals and aquatic organisms is on reproduction. Organochlorine (OC) pesticides has caused thinning of egg shell in several avian species and result in population declines (Risebrough, 1986). The adverse effect on the fish reproductive cycle has also been reported (Burdick et al., 1967). In case of carbamates and organo phosphorus compounds, acute inhibition of acetyl cholinesterase (AChE) activity leads to disruption of nerve function and ends in mortality (Ludke *et al.*, 1975; Hill and Fleming 1982). In order to assess the impact of insecticide spray programme on forest songbirds, the relationship between dosage of the pesticide and degree of inhibition of AChE has been studied (Mineau and Peakall, 1987). Pesticide exposure based behavioral changes - not particularly sensitive - has been observed in birds (Peakall, 1985). Terbufos and carbofuran, important insecticides for controlling corn rootworm larvae in soil are highly toxic to earthworms. Foliar applications of broad spectrum insecticides produce nearly total depletion of arthropod populations in crops such as cotton (Albert et al., 1988).

Pesticides enter natural water from direct application for control of aquatic weeds, trash fish, aquatic insects, percolation and run off from agricultural lands, drift from industrial waste water and discharge from waste waters from clean up equipment's used for pesticide formulation and application (Van Schoubroeck, 1989). The organo-chlorine groups of pesticides are most commonly found in surface waters, because they are not rapidly degraded in water (Lee et al., 1982; Barbash and Resek 1996). Cytological aberrations in plants serve as an excellent monitoring system for the detection of environmental chemicals that may pose a genetic hazard (Nilan and Vig, 1976). Exposure of pesticides to plants can disrupt photosynthesis, alter plant metabolism and reduce crop yields etc. The plants may absorb pollutants from water and pass them up the food chain to consumer animals and humans. The effects of the pesticides can be studied on certain biochemical constituents, physiology, growth and yield of test plants. If a pesticide influences the growth of the plant populations, then the food/energy supply for the ecosystem is reduced, lowering its productivity and stability (Yadav, 2007; 2010). Plants and other organisms may obtain some essential elements (C, H, O, N) from the atmosphere. Other nutrients are obtained from soil or water, and are cycled through the biota.

Pesticides may be capable of reducing the variability of one or more organisms involved in the recycling process in an ecosystem. To an extent the pesticides affects the decomposers such as microbes, earthworms, insects, etc., which are responsible for the decomposition of organic matter (Yadav, 2010).

An appreciable proportion of the insecticides applied often reach the soil either directly from deliberate applications to the soil or indirectly from runoff from leaves and stems of the plants. Persistence and dispersion of pesticides in the soil environment depends not only on the properties of the pesticides but also on the properties of the soil and the prevailing climatic conditions (Khan, 1980). The cycling of pesticides within soils is influenced by a combination of chemical, physical and biological processes. While processes controlling pesticide cycling in the organic part of soils are primarily microbial, control on pesticide retention within the mineral region is primarily controlled by adsorption to soil surfaces (Kalbitz et al., 2000). Adsorption is rapid, occurring within seconds to minutes, and thus occur more rapidly than microbial decomposition (Qualls and Haines, 1992). Quantifying these sorption mechanisms will help to understand whether the pesticides are retained in soil or are released to the soil environment for further action. There are various factors which play a role in pesticide persistence and degradation in a particular region. Soil characteristics and microbial flora play crucial roles in the fate of pesticides. The intensive use of pesticides in agriculture may cause contamination of ground water resources due to their leaching through the soils into aquatic regions. There are various reports on the ground water level of pesticides in the temperate regions (Ritter, 1990). Studies from the different tropical regions have shown that pollution of ground water may be of concern in tropical regions, too (Lanchote et al., 2000; Li et al., 2001). Currently there are a number of possible mechanisms for the clean-up of pesticides in soil, such as chemical treatment, volatilization and incineration. Most of these physical-chemical cleaning technologies are expensive and rather inefficient (Kearney, 1998; Nerud et al., 2003) because the contaminated soil has to be excavated at a site and moved to a storage area where it can be processed. For this reason several biological techniques involving biodegradation of organic compounds by microorganisms have been developed (Schoefs et al., 2004). To assess the risk of ground water contamination by pesticides, their persistence and mobility in soil need to be determined (Roberts, 1996). Because of the alteration in different climatic conditions, lab studies may not be adequate to establish the pesticide persistence and mobility under outdoor conditions (Laabs et al., 2002).

At present, India, with an annual production of 90,000 tons, is the largest producer of pesticides in Asia and ranks twelfth in the world in the use of pesticides (Gupta, 2004). Pesticides being used in agricultural tracts are released into the environment and come into human contact directly or indirectly. Humans are exposed to pesticides found in environmental media (soil, water, air and food) by different routes of exposure such as inhalation, ingestion and dermal contact (Yassi et al., 2001). There is a dearth of studies related to these issues in India. A study which looks into the health effects of acute pesticide use among the cotton growers of India by Mancini (2005) is a positive step to fill this research gap. Another recent report has discussed in detail about the pesticide use and application in the Indian scenario (Abhilash and Singh, 2009). The use of synthetic pesticides started in 1948–49 with the use of DDT for malaria control and BHC for locust control. Orgnochlorine insecticides, such as DDT, hexachlorocyclohexane (HCH), aldrin and dieldrin, are among the most commonly used pesticides in the developing countries of Asia because of their low cost and versatility against various pests. Even in the 1990s more than 70% of the gross tonnage of pesticides used in agricultural applications in India consisted of formulations which are banned or severely restricted in the east and west (Abhilash and Singh, 2009). India is one of the few countries still engaged in the large scale manufacture, use and export of some of the toxic chlorinated pesticides, such as p,p'-dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH) and pentachlorophenol (PCP) (Shetty 2001). Specific studies dealing with the agricultural practices of the farmers regarding pesticide use and its health impacts is needed to make informed policy decisions to bring about changes in the agricultural practices in India. The various works carried out nationally and internationally, by different researchers gives a clear glimpse of the adverse effects of pesticides on environment.

Assessing the toxic effects of pesticides on ecosystems is difficult because of the complexity of the interactions between several species and processes. Some pesticides exert their effects on particular components of an ecosystem, for instance, some herbicides affect primary production in plants, and persistent insecticides bio-accumulate in higher tropic levels such as predators (Yadav, 2007; 2010). In order to assess the complete impact of pesticides on an ecosystem, we need to address various components such as persistence of chemicals in terrestrial and aquatic environment, role of soil characteristics and microbial flora on pesticide degradation etc.

Fate of Pesticides in Soil

Soil is defined as the "collection of natural bodies occupying portions of the earth's surface that support plants and that have properties due to the integrated effect of climate and living matter, acting upon parent material, as conditioned by relief, over periods of time"(Natural resources conservation service may 2006). Soil provides ecosystem services critical for life: soil acts as a water filter and a growing medium; provides habitat for billions of organisms, contributing to biodiversity; and supplies most of the antibiotics used to fight diseases. Humans use soil as a holding facility for solid waste, filter for wastewater, and foundation for our cities and towns. Finally, soil is the basis of our nation's agroecosystems which provide us with feed, fiber, food and fuel.

Pesticides are poisons designed to kill pests such as rodents, insects, weeds and fungi. Pesticides are, by their nature, toxic chemicals; since many pesticides may potentially leave residues on foods available for human consumption, there is much concern regarding the potential health risks of pesticides in the human diet. Pesticides used in agriculture to control pests, such as insects, weeds, and plant diseases, have been subject to considerable legislative, regulatory, and consumer scrutiny over the past few decades. Pesticides, with their high degree of toxicity, constitute a very important group of target compounds in environmental samples. Those present in waters may have an agricultural, domestic or industrial origin, the most harmful effect being their inclusion in the so-called "nutrition chain" (Vinas et al., 2002). Many common pesticides contain potent neurotoxic chemicals that attack and disable portions of the nervous system and brain. The use of pesticides in commercial agriculture has led to an increase in farm productivity (Guler et al., 2010). Pesticides also present environmental concerns including water and soil contamination, air pollution, destruction of natural vegetation, reductions in natural pest populations, effects upon non-target organisms including fish, wildlife, and livestock, creation of secondary pest problems, and the evolution of pesticide resistance (Winter, 2004). Many pesticides were used on a global scale from the 1950s to the mid-80s, most of which are stable and persistent in the environment (Barra et al., 2001).

According to the World Health Organization (WHO), approximately three million people are intoxicated each year as a result of the use of pesticides. In addition to the toxicity to humans, the presence of these products in the environment poses a risk to

water quality and the ecosystem (Veiga et al., 2006). When applied to the soil, they can reach other levels as a result of mobility, sorption, volatilization, erosion, and leaching, thereby contaminating various environments (Andreu and Pico, 2004; Nawab et al., 2003).

Pesticides may reach the soil through direct application to the soil surface, incorporation in the top few inches of soil or through the unauthorized dumping of unwanted pesticide products. Pesticides may enter ground water sources and surface run-off during rainfall, thereby contributing to the risk of environmental contamination. All pesticides are subject to degradation or metabolism once released into the environment. Intensive agricultural practices often include the use of pesticides to enhance crop yields. However, the improvement in yield is associated with the occurrence and persistence of pesticide residues in soil and water. Kerala, being the largest production center of cardamom in India and cardamom the highest pesticide consuming crop, the risk of environmental contamination especially in soil and water is high (Susan and Reshmi, 2014).

Groundwater is an essential and vital component of our life support system. The groundwater resources are being utilized for drinking, irrigation and industrial purposes. There is growing concern on deterioration of groundwater quality due to geogenic and anthropogenic activities. Groundwater, being a fragile must be carefully managed to maintain its purity within standard limits. Groundwater degradation occurs when its quality parameters are changed beyond their natural variations by the introduction or removal of certain substances (Ramesh, 2001).

A pesticide that has not become a gas (volatilized), or been absorbed by plants, bound to soil, or broken down can potentially migrate through the soil to groundwater. Groundwater movement is slow and difficult to predict. Substances entering groundwater in one location may turn up years later somewhere else. A difficulty in dealing with groundwater contaminants is discovering the pollution source when the problem is occurring underground, out-of-sight. Also, microbial and photodegradation (by sunlight) do not occur deep underground, so pesticides that reach groundwater break down very slowly. Quality assessment and management are to be carried out hand-in-hand to have a pollution free environment and for a sustainable use (Kannan et al., 2010).

Pesticide leaching can also be indirectly affected by changes in the agro ecosystem that are triggered by climate change, changes in land-use, modified pesticide application timings or the use of different pesticides against invasive weeds, diseases or pests (Bloomfield et al., 2006; Whitehead et al., 2009). The fate of pesticides in soil and water environments is influenced by the physio-chemical properties of the pesticide, the properties of the soil and water systems (presence of clay size particles, organic matter and pH), climate, biology, and other factors (Martı´nez-Carballob, 2007). The rates of degradation and dissipation may vary greatly from pesticide to pesticide and situation to situation. The solubility of common pesticides in soil and water is very low and its half-life period very short (Mathavakumar et al., 2006). There are very few references or studies on pesticide residues in elevated areas covering large hilly regions where the environment is exposed to frequent application of toxic pesticides.

Increased rate of agricultural activities during the last many decades have deteriorated the soil entity and ground water quality of Kasargod district, Kerala. As a consequence, the study on both the soil and water quality of the district has become paramount priority for contamination assessment programme. Thus the soil characteristics in Kasargod district was carried out by collecting 11 soil samples from high land mid land regions. Trace metals (Fe, Ni, Mn, Mg, Co, Zn, Cu, Pb and Cd) as well as organochlorine pesticides in soil were analysed concurrently. The observation resulting from the study signifies that, most of the area is contaminated by Pb, Ni and Co (Jyothish et al., 2013).

The use of pesticides has proved to be the only means to protect crops on a large scale. However, the effects of pesticide usage must be seen also in the context of soil pollution and sustainability of the agro ecosystem. Some crops, such as cotton, need heavy repeated applications of different pesticides, including organophosphates, carbamates, pyrethroids, and organochlorines which reach the soil. All the biological parameters varied each sampling time and values also varied among soil samples, being inhibited or stimulated by the different pesticide applications, but they mostly recovered the initially detected activity (Andrea et al., 1999).

The agricultural use of pesticides, in particular herbicides, has often led to groundwater pollution; the interaction of these substances with soil, plays a major role in determining the occurrence and the extent of groundwater contamination. The persistence of organochlorine pesticides in different environmental matrices is a

matter of concern as the complete environmental fate of these chemicals is still an unexplored field. A recent study provides information on the current residue levels and persistence of organochlorine pesticide endosulfan in water, sediment and soil in selected areas of Kasaragod district. The study shows that combined toxic residues of endosulfan in the sediment and soil samples were found to be persistent for a period of 1.5–2 years, but the persistence showed variations depending upon the climatic conditions and physico-chemical characteristics like pH, organic matter content and particle size of the soil in the area (Harikumar et al., 2014).

The retention of a pesticide by soil can prevent its short-term access to ground or surface waters and its effects on non-target organisms, but the persistence of the under graded pesticide or of harmful metabolites constitutes an ever present – and cumulative – risk to the environment and, eventually, to human health. The soil mineralogy, chemical decomposition and atmospheric conditions highly influence the pesticide retention and release of it and consequently the degradation of the compound. However, the indiscriminate use of pesticides in cardamom plantations will be a threat to the Cardamom Hill Reserves since the persistent pesticide residue of endosulfan, DDT and organophosphorous toxic chemicals are present in high concentration. The soil mineralogy of Idukki is favorable to its persistence and hence strict control and awareness to farmers for judicious use of pesticides is necessary to avoid pollution of water sources and contamination of the soil (Susan and Reshmi., 2014).

Many of the chemicals used in pesticides are persistent soil contaminants, whose impact may endure for decades and adversely affect soil conservation (U.S. Environmental Protection Agency, 2007). A smaller content of organic matter in the soil increases the amount of pesticide that will leave the area of application, because organic matter binds to and helps break down of pesticides (Kellogg et al., 2000).

Pesticides can contribute to air pollution. Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating environment (Kellogg et al., 2000). Volatile pesticides applied to crops will volatilize and are blown by wind to nearby areas posing a threat to wildlife. (Reynolds, 1997). Sprayed pesticides or particles from pesticides applied as dusts may travel in the wind to other areas, or pesticides may adhere to particles that blow in the wind, such as dust particles (National Park Service, 2006). Compared to aerial spraying, ground spraying produces less pesticide drift. Farmers

can employ a buffer zone around their crop, consisting of empty land or non-crop plants such as evergreen trees to serve as windbreaks and absorb the pesticides, preventing drift into other areas and thereby less pollution.

Pesticides have more negative impact on our ecological system when compared to its desired action. Pesticides are also causing water pollution and some pesticides are persistent organic pollutants which contribute to soil contamination (Rockets Rusty, 2007). The amount of pesticide that migrates from the intended application area is influenced by the particular chemical's properties: its propensity for binding to soil, its vapor pressure, its water solubility and its resistance to being broken down over time (Tashkent, 1998). Some pesticides contribute to global warming and the depletion of the ozone layer.

Pesticides were found to pollute every source of water including wells (Gilliom et al., 2007). Pesticide residues have also been found in rain and groundwater (Kellogg et al., 2000). Pesticide impacts on aquatic systems are often studied using a hydrology transport model to study movement and fate of chemicals in rivers and streams. Studies by the UK government showed that pesticide concentrations exceeded those allowable for drinking water in some samples of river water and groundwater (Bingham, 2007).

Pesticides can enter the human body through inhalation of aerosols, dust and vapor that contain pesticides; through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with skin (Department of Pesticide Regulation, 2008). Pesticides are sprayed onto food, especially fruits and vegetables, they secrete into soils and groundwater which can end up in drinking water, and pesticide spray can drift and pollute the air.

To assess the leaching potential of pesticide, traditional concept mainly focuses on chromatographic flow processes that are dominant in sandy soils. For these soil types, the computer models work rather well and allow meaningful adaptation of outdoor studies to other climatic conditions based on modeling. However, chromatographic transport through the soil matrix system is not the only process that has to be considered when predicting the leaching of chemicals. Especially in structured soils (silty and/or clay soils) preferential flow through macropores formed by shrinking cracks and fissures becomes the dominant leaching process. To study macropore transport in more detail, lysimeters were used. Computer models

to simulate pesticide transport in the unsaturated zone will be an important management tool in coming years. However, not many efforts were directed to validate the usefulness of such models. The models (GLEAMS, PRZM, PELMO and LEACHM) use different mathematical concepts to compute one-dimensional flow and transport in a homogeneous, unsaturated soil. The model simulations were based on standard laboratory measurements of the physical and chemical soil-pesticide properties of the sample soils and pesticides. Among the three models, PRZM and LEACHM performed best, while GLEAMS computations are fast at the cost of accuracy.

Detection and Decontamination of pesticide residues

Several different technologies available for the treatment of pesticide contaminated sites have been reported (De - Wilde et al., 2007; Shaalan et al., 2007; Chaudhry et al. 2005; Aitken and Long, 2004; Wait and Thomas, 2003). Bioremediation process can be divided into three phases or levels. First, through natural attenuation, contaminants are reduced by native microorganisms without any human augmentation. Second, bio-stimulation is employed where nutrients and oxygen are applied to the systems to improve their effectiveness and to accelerate biodegradation. Finally, during bioaugmentation, microorganisms are added to the systems. These supplemental organisms should be more efficient than native flora to degrade the target contaminant (Salinas-Martínez et al., 2008).

Microorganisms are considered to be the principal agents for the degradation of pesticides in bioremediation processes. Considering that the earth is the home for an uncountable species of microorganisms, the pesticides applied to these soils probably undergo an accelerated degradation by these organisms. Wyss et al., (2006), were able to remove the pesticide endosulfan from the environment. They isolated 16 microorganisms from the soil for this purpose. "*Aspergillus*" of the fungi kingdom totally removed endosulfan after incubating for 12 days with the pesticide. The levels evaluated were between 35.0 and 350.0 $mg.L^{-1}$. The results demonstrated that *Aspergillus* is a potent and easily acquired bio remediating agent that could be used to remove other pollutants from water and even soils.

Wyss et al., 2006, isolated and characterized the bacterium *Pseudomonas sp* for the hydrolysis of atrazine. This microorganism used atrazine as a nitrogen, citrate and carbon source, and for the production of electron donor molecules under aerobic conditions. The degradation of approximately 100.0 $mg.L^{-1}$ of atrazine occurred. The

authors observed that concentrations of atrazine above 100.0 $mg.L^{-1}$ were toxic to *Pseudomonas* sp. Giacomazzi & Cochet, 2004, studied the transformation of the herbicide diuron in water by microorganisms present in the soil. The reaction was catalyzed by OH- and H+ with organic and inorganic matter from soil dissolved in the aqueous phase. The proposed system presented good results for the chemical degradation of diuron. The authors suggest that microorganisms could be used to promote the biological treatment of polluted sewage water.

Photocatalytic oxidation is a very advanced process used to remove and degrade pesticide residues from various environments such as soil, water and food. In recent years, several studies and reviews on advanced oxidative processes (AOP) (Wu et al., 2007; Lasa et al., 2005; Carp et al., 2004), which use UV light. Titanium dioxide is used extensively in most of these studies and is one of the most extensively used processes among those mentioned because it is a readily available reagent, chemically robust and durable (Legrini et al., 1993; Chen and Ray, 1998; Leyva et al., 1998; Kabra et al., 2004; Oh et al., 2004; Canle-Lopez et al., 2005). Moctezuma et al., 2007, studied the photocatalytic degradation of methyl parathion pesticides, using TiO_2 in aqueous suspension. The final products were phosphoric acid and CO_2. Furthermore, Mahalakshmi et al., 2007 demonstrated that the combination of TiO_2 and ZnO_2 was very effective in the photocatalytic mineralization reactions of carbofuran in water samples under solar radiation. They also assessed the total quantity of organic carbon (TOC) to confirm the extent and effectiveness of the mineralization process used in this study. Four intermediate products of the carbofuran were formed after only six hours of reaction. Various pathways for degradation of this compound were proposed.

The analysis of pesticides in soils, animals and plant tissues, the establishment of residue tolerance in food, and environmental studies involving trace contaminants are all dependent on trace analytical method employing gas chromatography (GC). Advances in this techniques are closely related to the development of the more sophisticated analytical procedures employed today. Investigations in the early 1950s with gas phase separation technique reveled unparalleled resolution of complex mixture of compounds such as those resulting from extract of natural matrices. However at that time, the measuring devices used to monitor eluting components were relatively insensitive. These early detectors measured changes in thermal conductivity or gas density of the effluent using hot wire filaments and were limited to the detection of only 0.01 M in the effluent exploitation of ionization

technique in detector designs in the late 1950s resulted in lower detection limits and greatly extend the application of gas chromatography.

The identification of pesticides based on the retention times of chromatographic peaks was mentioned by Bevenue (1963). Since positive identification is usually not possible from peak retention times alone, confirmation of identity is made by various other techniques. These methods (chromatographic and non chromatographic) when used in combination can provide univocal identification in many cases. One problem is that chromatographic detectors are very sensitive and only very small amount of pesticides are detected so that a sufficient amount of material may not be present for some of these auxiliary methods to be applied. It is especially difficult to identify multiple insecticide residues in a single sample from retention time on a single column. A combination of procedures, however, allows the evaluation and identification of multiple residues in sample having an unknown or incomplete history of treatment. Methods were developed by combining column chromatography for the fractionation of compounds difficult to resolve by gas chromatography with chemical conversion of the parent insecticides to alternate gas chromatography responsive products and two column gas chromatography for their special identification in large number of samples of soils, alfalfa, carrot, corn, tobacco etc. The combination of a gas chromatograph with a mass spectrometer provides an instrument with the maximum potential for the analysis and precise qualitative identification of complex mixtures. Much effort is being expended to achieve the maximum potential of this combination for combinations of various types.

Because of the potential for long-term damage to critical structures such as the nervous system, immune system and endocrine system, prevention of all acute poisoning events as well as of exposure to low doses during development is a high priority for ensuring human and environmental health. There are many steps that can be taken at the local/practice level, national/government level and international treaty/trade levels to decrease exposure to pesticides and related illnesses. Many organizations (especially FAO and WHO) promote alternative non-chemical forms of pest-control and there is an increasing engagement in non-pesticide dependent agriculture and integrated pest management (IPM). Education is a key component of safe pesticide use and prevention of toxic exposures. Farmers, pesticide applicators and their families need to be informed and educated on how to recognize and prevent pesticide poisonings. Trained or licensed pesticide applicators can maximize

preventive measures Nine of the 12 persistent organic pollutants (POPs) included in the Stockholm Convention, are pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene and hexachlorobenzene). Other international organizations such as the Intergovernmental Forum for Chemical Safety (IFCS), the International Labour Organization (ILO) and others are working towards safer use and monitoring of pesticides at the global level.

Study area

Cardamom Hill Reserves (CHR), Idukki, Kerala

Study Area

Cardamom Hill Reserves (CHR) is situated in the South Western Ghats, Idukki District of Kerala, India at 76^0 58′ E to 77^0 16′ E and 9^0 37′ N to 10^0 2′ N. In Idukki District, about 97 % of the total area is covered by undulating hills, rugged mountains, forests and valleys. There are around 11 mountain peaks which exceed 6000 ft in height, including Anamudi, the highest peak in Kerala (8,842 ft). Idukki comprises a geographical area of 5,14, 962 ha, of which about 50% is Reserved Forests. Among Kerala's population, 3.7 % occupies this space. About 66% of the electric power of the State is generated here. The district is divided into four revenue taluks viz., Devikulam (1774.2 sq. km), Peerumedu (1286.4 sq. km), Udumbanchola (1071.4 sq. km) and Thodupuzha ((973.3 sq. km), 64 revenue villages, and eight developmental blocks, 51 panchayats and one municipality.

Based on the topography and altitudinal range, the land in Idukki district can be classified in to five categories; midland 20 -100 m above MSL), mid-upland (100m - 300m above MSL), upland (300m - 600m above MSL), Western Ghats high range (600m - 1200m above MSL) and top Western Ghats high range (>1200m above MSL). The high ranges occupies the altitude range from 600 m to more than 1600 m ft above MSL. Diverse climate and topography in the region promotes assorted flora and fauna. The district mainly has high land covered by rugged mountain ranges, hills and deep valleys, which covers up to 96 % of the total area. Different types of forests seen in the district are (1) moist deciduous forests, (2) evergreen/semi-evergreen forests, (3) dry deciduous forests, (4) grasslands, (5) mountain sub-tropical/temperate forests, and (6) forest plantations.

The climate of the region varies with altitude, land pattern and forest coverage. Moderate climate prevails in the midland area with temperature varying between 21^{0}C to 30^{0}C and having minimum seasonal variation. The average rainfall is 3500 mm with variation from 2500 to 4500 mm and rarely going up to 7000 cm. The economy of Idukki district mainly depends on agricultural income. The district has a gross cropped area of 2,98,662 ha. The distinctive terrain, agro-climatic conditions and terrain of the district are most appropriate for growing plantation crops. The district is prominent for cultivation of largest area under various spices, particularly small cardamom, and contribute a large share to the total spice production in the state.

The small cardamom, *'Elettaria cardamom'* is a crop native to the region where this species along with related species were growing in wild state. The area within Idukki district, where cardamom was growing in wild state and found ideal for its cultivation is designated as Cardamom Hill Reserve (CHR) forest.

According to government declaration in 1897 CHR forest spread across hills with altitude between 600 and 1200 m above MSL in the Devikulam, Udumbanchola and Peerumedu taluks of Idukki (Figure-1). Lower elevation (600 - 800m) of CHR is mainly composed of plantations such as cardamom, rubber, cocoa, coffee, coconut, arecanut, etc. Intensive cardamom cultivation is mainly confined in the altitude ranges from 800-1200 m above mean sea level (MSL). Forest loam is the main soil type in this area and is acidic in nature. Annual rain fall ranges from 1500 mm to 6000 mm, of which 60 -40 % is from south western monsoon. Temperature ranges from 15^0 C to 36^0 C. In Cardamom Hill Reserve, Small Cardamom (*Elettaria cardamomum* Maton) is cultivated as an under storey crop in the tropical ever green forests in the altitude ranging from 500-1500m above MSL with an average annual rainfall between 1500 to 6000 mm.

The Cardamom Hill Reserve forest is known for best agroecological conditions for cardamom cultivation with evergreen forest cover and well spread high rainfall. Cardamom is very sensitive to moisture and light stresses and needs cool and shady evergreen forest environment for better performance. Cardamom growing areas falls under CHR region are reported to offer highest average cardamom yield compared to many other areas. For sustained cultivation and yield of cardamom, conservation of forest cover is important.

Soils in the cardamom hills are primarily Ultisols on granitic bedrock with loam to loamy sand surface texture. Argillic and, in some cases, lateritic horizons are present. Soils in valleys are deep (frequently more than 5 m) while on ridges and crest they are shallow up to 1 m. Until early 1950s, the cardamom hills had dense tropical mixed forest and shade pruning was infrequent. In the recent past decades regular and severe shade lopping has been practiced by almost all the growers of cardamom.

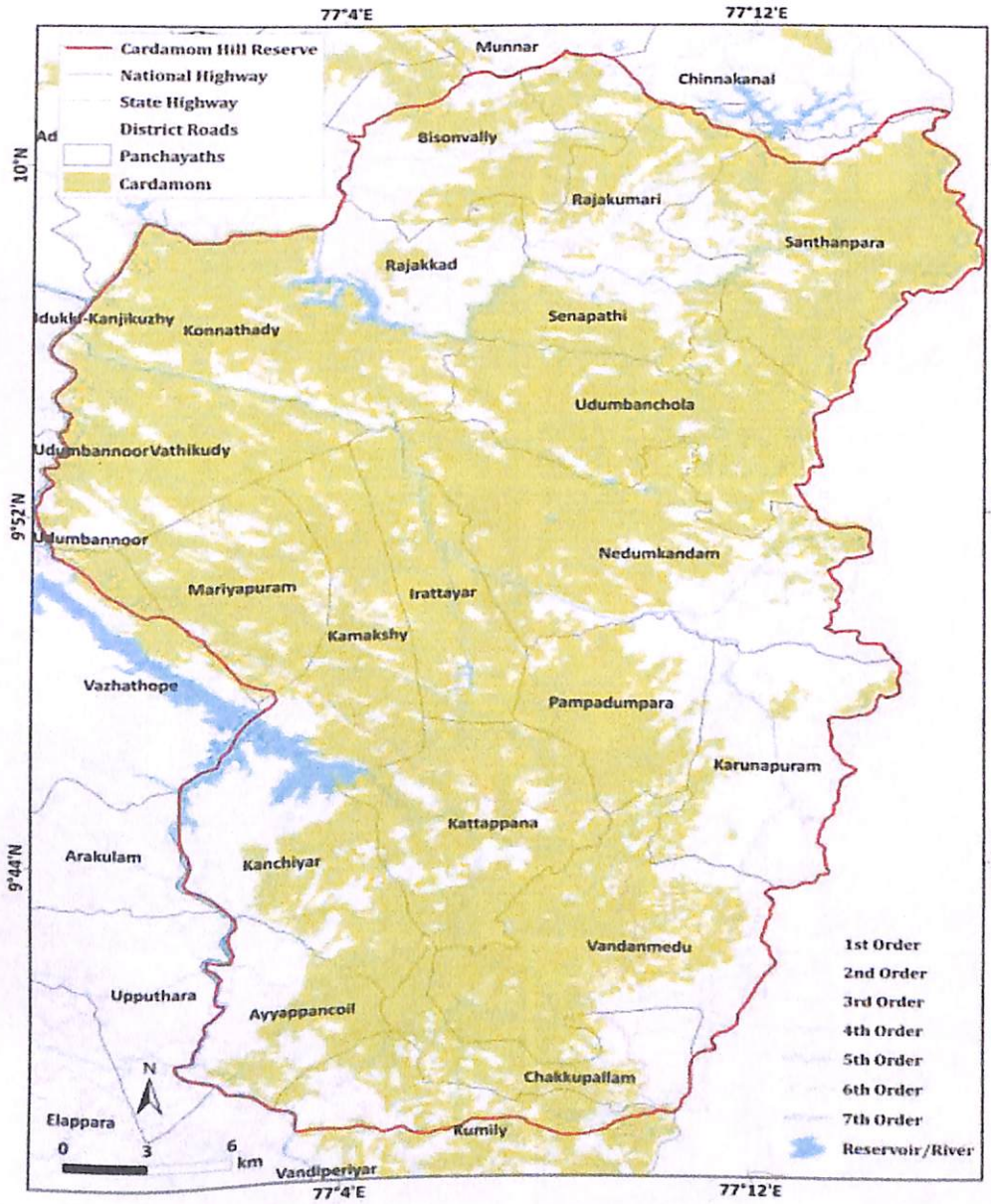


Figure-1 : Area under Cardamom Hill Reserves.

The total study area covered under the present study is 873 km² and this area was equally divided in to 198 grids (Figure-2). Area covered under each grid was 2.5 km². The sample collection was carried out based on randomized block design (RBCD) method. Data and soil samples were collected from every 9th grid. Accordingly, a total of 24 grids were randomly selected for sampling. For analysis of soil characteristics and pesticide residues, soils were collected from 4 random spots in the selected grid, with four replicates at each spot. The soils

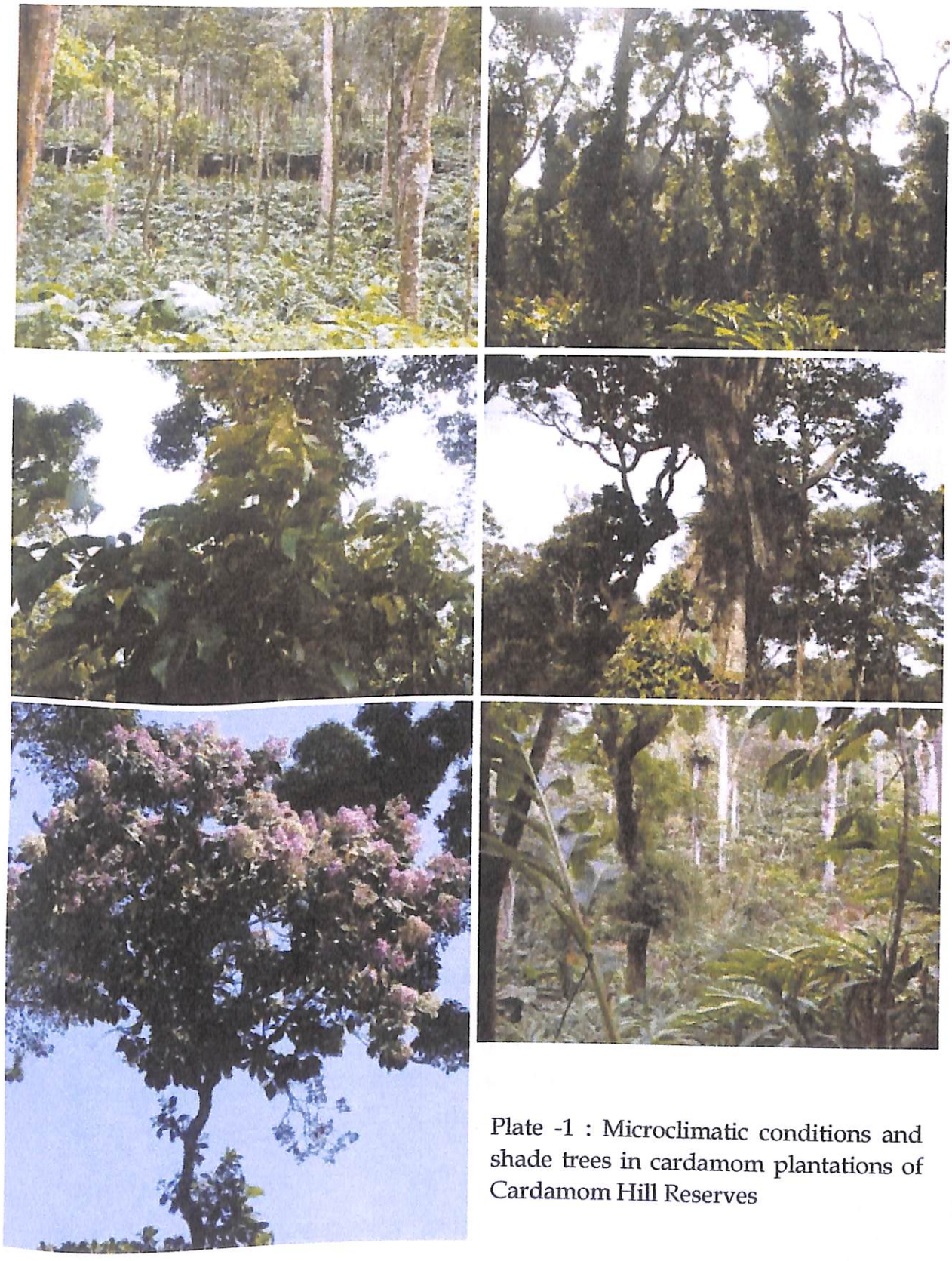


Plate -1 : Microclimatic conditions and shade trees in cardamom plantations of Cardamom Hill Reserves

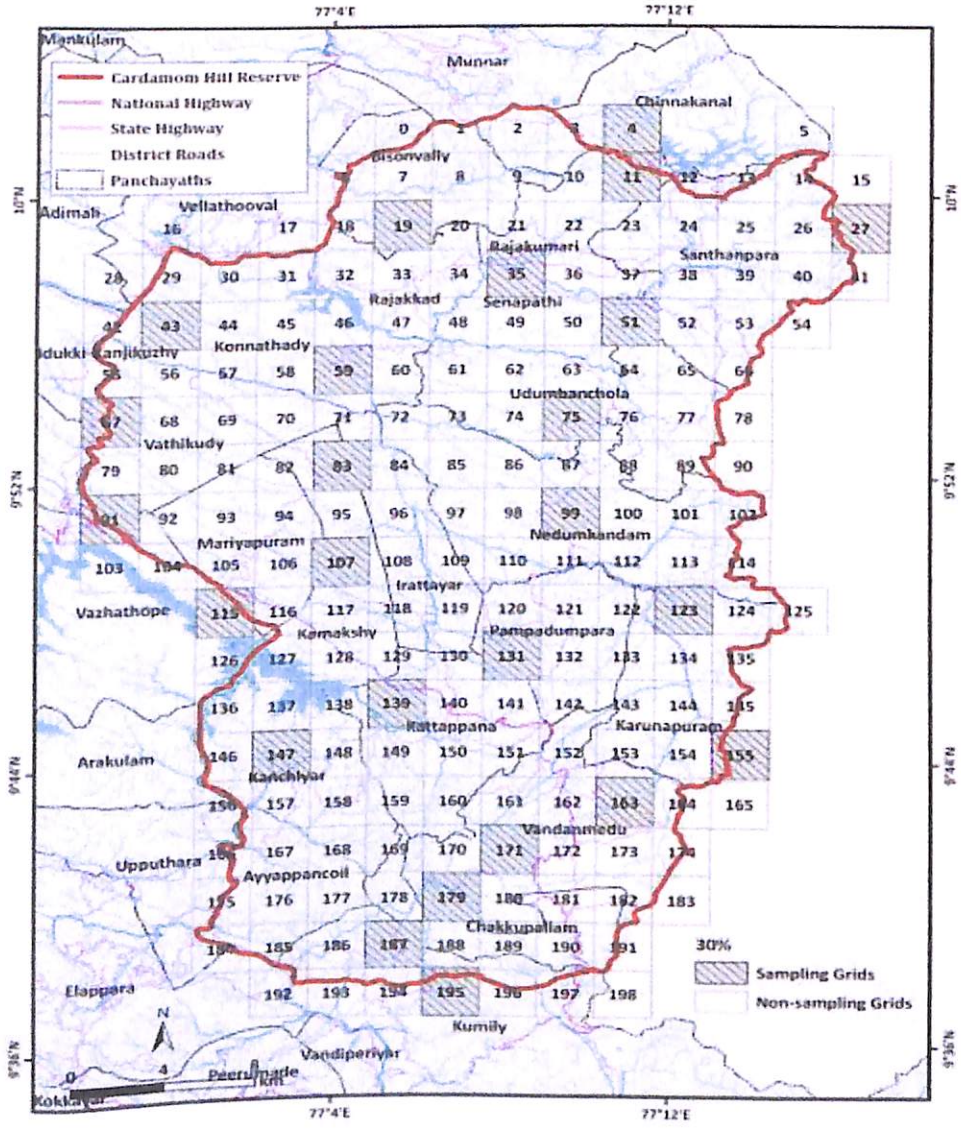


Figure - 2 : Block design of the study area

were collected from two depths; 0-20 and 20-40 cm. For microbial isolation, soil samples were collected from same locations but the depth was restricted to 0 -10 cm (depth of maximum microbial activity) and mixed together to get one composite sample. The samples were air dried and stored at 4°C for further studies.

Experiments, Results and discussion

Section -1

Pesticide use in Cardamom Hill Reserves and their biochemical toxicity

1. Introduction

The major hazards in crop production are pests and diseases which require intensive management. Each year pests destroy almost half of the world's food crops. This leads to heavy use of chemical agents to protect the crops. Serious environmental concerns are reported due to indiscriminate use of pesticides in agriculture for crop protection. The use of pesticides has aided substantially to decrease crop losses and to get better yield of the crops. On other side, their unfavorable effects in the form of environmental degradation and human health go unnoticed. The main concern of pesticide mismanagement starts with severe damage to soil, water, human health and the environmental degradation (Huber et al., 2000; Kidd et al., 2001; Ntow, 2001; Cerejeira et al., 2003). More than 85 % of the pesticides used are applied in agriculture (Aspelin, 1997)

In addition to polluting soils and water, pesticides also persist in the crop products, thus entering the food chain, and enter the animal body system, blood and organs. The pesticides also contribute to environmental pollution, biodiversity losses and deterioration of natural habitats (Cerejeira et al., 2003). In most of the cardamom plantations, insecticides are applied at regular and short intervals (20 to 25 days) regardless of pest incidence, with the only aim of producing capsules free from scars and bore holes and thus making the pest management costly and sometimes ineffective. As the end users, farmers apply higher concentrations of pesticides at increased frequency and also combines several pesticides together in order to combat pesticide resistance (Chandrasekhara et al., 1985; WRI, 1999).

Pesticides causes both short and long-term ill effects on human health. As per a United Nations study about 2 million poisonings and 10,000 deaths occur each year from pesticides, with about three-fourths of these occurring in developing countries (Quijano, 2002). In many cases pesticides used in developing countries were not fully tested for their toxic effects and testing in the past has focused on acute effects rather than long-term effects.

2. Objectives

The major objectives of the present study are

1. Identifying the major pesticides being used in the region, their chemical classification and extent of use
2. Qualitative and quantitative analysis of pesticide residues in soil
3. Study the biochemical toxicity of selected pesticides on soil invertebrates.

3. Materials and Methods

3.1 Survey

Survey was conducted among the cardamom growers (96 Nos) to evaluate the details of pesticides used, nature of chemicals, quantity, pattern of use etc. Data pertaining to land holding size, land use pattern, fertilizer use, major pests and reasons for pesticide application were also collected. Discussions were carried out with cardamom planters to understand the extent of use and criteria for selecting specific pesticides.

3.2 Collection of soil for residue analysis

Composite soil samples from cardamom cultivated areas as well as natural cardamom growing areas, were collected during the pre-monsoon months (March-May) and post monsoon (August - September) months. Soil samples of two depths 0-20 cm and 20-40 cm were collected separately, air-dried, cleaned, pulverized and sieved (2 mm sieve). The sieved soil samples were properly stored in polyethylene bottles and used for further analysis

3.3 Pesticide residue analysis

The gas chromatography coupled with mass spectrometry method was employed to determine the concentrations of pesticides in soil samples. This multi residue analysis of pesticides was performed as per the procedure given in the AOAC (Association of Official Analytical Chemists) guidelines. In the present study, 30 g of soil was weighed in to a 250 ml conical flask. Twenty ml of chloroform (sufficient to sock the soil completely) was added and mixture was vortexed for 30 minutes. Centrifuged and collected the supernatant. Repeated the step 3 times. Transferred the extract to beaker and allowed to reduce the volume of extract to 1/10 by using air purge. Concentrated sample were filtered through 0.45 micron syringe filters (Ambrus et al., 2005).

The extracts of pesticide residues in soil samples extracted by chloroform extraction method were analyzed by GC-MS. One micro liter of the extract was injected through split less injector at 250°C temperature, on the GC-MS for analysis. Helium was used as carrier gas (1.2 ml/min). Extracts were analyzed with a Shimadzu QP2010 series GC-MS instrument. Electron ionization was applied in the MS, which typically run in the scan mode. Temperature in this injection port allows the conversion of liquid in to gas. GC-MS Real time analysis software was used for instrument control and data analysis. MS conditions: Ion source Temperature - 200^{0}C, Interface Temperature - 250^0 C and GC conditions:

Column Oven Temperature - 60^{0}C, Injection Temperature - 250^{0}C, Injection Mode - Split less (Luke et al., 1981).

3.4 Soil mitochondrial dehydrogenase activity

The experiment was conducted for determination of microbial activity of soils and direct effect of chemicals or pesticides on non-specific soil microbial community. Concentration of soil dehydrogenases depends on conditions and intensity of biological conversion of organic compounds. Addition of Triphenyltetrazolium chloride (TTC) enhances bioavability of endogenous soil organic compounds to microflora. At the same time chloride is converged by hydrolytic reaction to Triphenyl formazan which can be extracted by organic solvents (methanol, acetone). Formazan concentration can be determined spectrophotometrically at 485 nm.

1 g air-dried soil was taken in 15 ml test tubes. Added 0.4 ml of 3% TTC solution in each of the test tubes to saturate the soil followed by addition of 0.75ml of 1% glucose in each test tube. Covered the mouth of each test tube using Parafilm. Incubated the tubes at 28 ± 0.5^{0}C for 24 hrs. After incubation 5 ml of methanol was added and shaken vigorously. Allowed to stand for 6 hrs. Vortexed the mixture at intervals of 1 h. After 6 hrs, the solution was centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and measured at 485 nm against a standard curve created with different concentrations of Triphenyl formazan (TPF). The dehydrogenase activity was expressed as µg of TPF formed/gram of soil. The experiment was conducted with four replicates per grid after pooling the soil samples from each grid.

3.5 Toxicity Studies

Toxicity studies with most used organophosphate pesticide in the region - chlorpyrifos - was carried out using earthworms as model organisms. The studies were carried out in order to find out the toxicity of the chemical agent to the soil organisms and delineate the toxicity mechanisms.

3.5.1 Soil samples

Natural soil samples -free of pesticide applications for more than 30 years - were collected from abandoned grassland field at Kerala Forest Research Institute campus at Thrissur, India. The physical and chemical properties of the soils were carried out and the results were shown in Table - 1.1

Soil Parameters	Values	Soil Parameters	Values
Sand (%)	80.0	P (kg/ha)	1.3
Silt (%)	13.0	K(kg/ha)	408.9
Clay (%)	7.0	Calcium(mg/ kg soil)	369.8
pH	6.2	Magnesium(mg/ kg soil)	332.5
Organic Carbon (%)	2.4	MBC(mg kg^{-1} soil)	628.4
N (kg/ha)	478.3		

Table 1.1. General characters of the experimental soil

3.5.2 Test species

Eisenia fetida was selected as the test species as recommended in standard guidelines (OECD 1994; ISO, 2008). The earthworms were collected from a culture at Department of Soil Science, Kerala Agricultural University (Thrissur, Kerala, India) which were maintained at an average 22 - 24^{0}C in dark containers with layers of dried plants and cow-dung manure. Compost, soil from garden and waste from University campus were used as food source. For experimental purposes, the earthworms were brought to the Biochemistry laboratory of Division of Forest Ecology and Biodiversity Conservation, KFRI.

3.5.3 Chemicals and Reagents

Chlorpyrifos used in the study purchased from Dr. Ehrenstorfer, Germany. For Protein estimation Bio-Rad DC Protein assay kit (Bio-Rad, Hercules, California, USA) was obtained from Bio-Rad. Thiobarbituric acid was obtained from SRL Chemicals, Mumbai. All other chemicals were purchased from M/s. Merck India Ltd, until otherwise mentioned.

3.5.4 Experimental Design

Avoidance behavior test

The avoidance behavior test was carried out based on the guidelines by the ISO protocol 17512-1 (2008) and OECD (2004). Test was carried following standard methods (Loureio et al., 2005; Natal-da-luz et al., 2004), briefly, plastic boxes each one divided in to two equal sections using a divider, were used for the experiments. One half of the box was filled with chlorpyrifos of desired concentration in soil uniformly distributed and other half remained as control soil. Every test concentration (n = 6 doses - 0, 10, 20, 40, 80 and 100 mg per kilogram of soil) was run in four replicates, each one in a different box. After 24 h, 5 adult earthworms were placed in the middle of the soil surface. After 48 h, the divider was inserted and the earthworms in both sides were counted. The results of the

counting were expressed as net response (NR) in percentage according to ISO (2005). NR = C-T/N x 100 where C- Number of observed worms in the control soil, T - Number of observed worms in the test soil, N - Total number of worms. A positive NR indicates avoidance of the treated soil whereas 0% or a negative value indicates a non-response or attraction to the pesticide tested (Amorim *et al.*, 2005).

Toxicity evaluation and mortality studies

Different doses of chlorpyrifos; 0, 10, 20, 40, 80 and 100 mg per kilogram of soil were taken for toxicity studies and four replicate fsor each doses were made. After 24 h, five earthworms each per replica were placed in the control and pesticide applied soils. The exposure time of the experiment was fixed as 0, 1, 3, 7 and 14 days. Weight loss and mortality were regularly observed on these days. At the end of each time points the specimen samples were processed for biochemical assays.

3.5.5 Biochemical assays

Lipid Peroxidation

Lipid Peroxidation was carried out in earthworm tissue samples. Malondialdehyde (MDA) occurs in lipid peroxidation and was measured in tissues after incubation at 95^{0}C with thiobarbituric c acid in aerobic conditions (pH-3.4). The pink colour produced by these reaction was measured spectrophotometrically at 532 nm to measure the MDA levels (Ohkawa et al., 1979)

Protein Estimation

Protein Estimation was carried out in earthworm tissue samples by modified Lowry's method using (Bio-Rad) DC protein assay kit (Bio-Rad, Hercules, California, USA) with BSA as Standard. The blue colour produced by proteins with copper tartarate and Folin's reagent measured colorimetrically at 750 nm (Lowry et al., 1951).

Histology

The histology of earthworm was studied adopting the routine paraffin method (Humason, 1979). After the pesticide exposure time points, control and experimental animals, were blotted free of mucus, washed thoroughly in physiological saline. Thereafter they were cut into two and put in a specimen bottle and fixed with Bouin's fluid for 12 hours before subjecting it to histological procedures of embedding in paraffin wax, sectioning and staining with haematoxylin eosin for microscopic observation. The stained slides were observed and photographed in a Leica research microscope and QWin imaging system.

3.6 Statistical analysis

All data are expressed as mean ± S.E. from four replicates per treatment. Data were analyzed by one-way ANOVA followed by Dunnet's test for comparison between control and treatment groups. The level of significance was set at $p \leq 0.05$. All the results shown in this article were obtained from at least three independent experiments with a similar pattern.

4. Results

The Cardamom Hill Reserves is in the territory of Forests in Western Ghats covering an area of 334 Sq. miles (86511 ha) located in the taluks of Peerumedu and Udumbanchola in Idukki district. Cardamom grows as a wild plant in the natural evergreen forests of the ecosystem in the elevation of 1000 to 1600 meters above mean sea level (MSL). The microclimate of the evergreen forests favored the growth of cardamom. Small natural openings in the forest patches were ideal in providing microclimate and ground conditions for the growth and nourishment of cardamom plants.

4.1. Pesticide use and analysis of residue levels

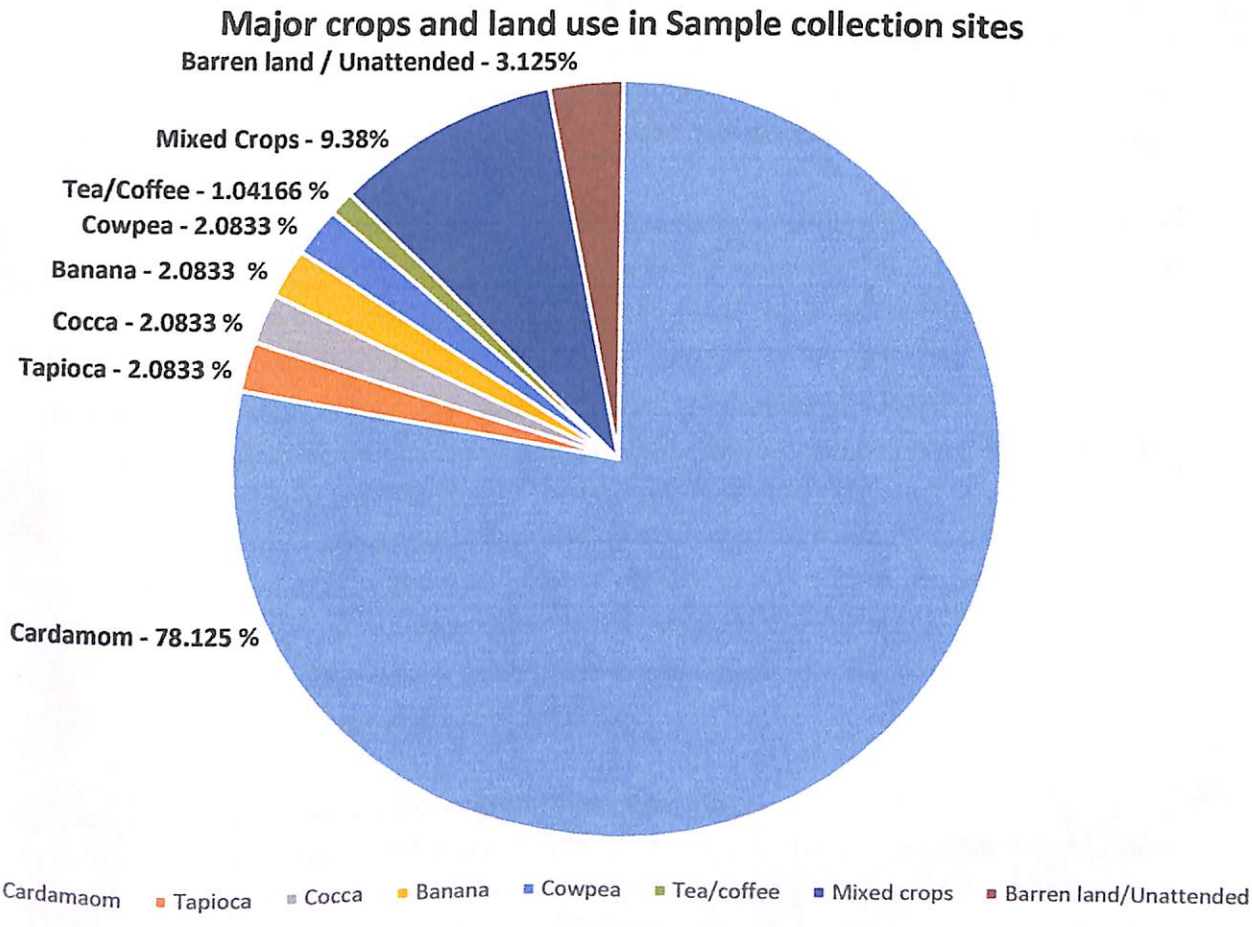
4.1.1 Major land use of Cardamom Hill Reserves.

Significance of the microclimate of tropical wet evergreen forest ecosystem in cardamom cultivation was realized by the early planters. Because of this, there was a thoughtful effort for the conservation of the ecosystem. The co-existence of rain forests and cardamom crops were a well-accepted practice in old times and regulations of those times were effective enough to take care of both the aspects. There were rules restricting the cutting and selling of trees from plantations, thus preventing the loss of floristic diversity and assuring the maintenance of ecosystem. Efficiency of cardamom production in CHR has been remarkable for the past 20 years (1990-2009) period with 4 fold increase from 200 kg to 800 kg ha^{-1}. Interestingly the cardamom cultivation area has come down drastically over the past three decades. CHR is a high biodiversity area under Western Ghats biodiversity hot spot and intensification of cardamom cultivation has negatively impacted the region.

Because of the high economic value of the cardamom, intensive agricultural practices are followed in the region. High chemical input in terms of fertilizers and pesticides is having a highly damaging impact on the ecosystem. Investigation of the questions for ecological impacts of exhaustive agriculture practices might reduce such practices and impacts. Present agricultural methods for rigorous

cardamom production include purposely upholding ecosystems in a highly simplified and nutrient rich state, however in a highly disturbed pattern.

Figure -1.1 – Major crops and land use in Cardamom Hill Reserves



The survey conducted in the study region showed the patten of land use as cardamom – 78.125 %, Tapioca – 2.08 %, Cocca- 2.08 %, Banana – 2.08 %, cowpea – 2.08 %, Tea/Coffee – 1.04 %, mixed crops 9.38 % and barren land/ unattended region – 3.125 % (Figure 1.1)

4.1.2 Agricultural practices of the region

Interaction with farmers of the region has revealed many of the practicing agricultural procedures in the region. Selection of the variety is one major criteria, varieties were selected by planters to suit to local cultivation conditions. At present "*Njallani*" is the most preferred cardamom variety in the region and are cultivated by most of the farmers because of its high yield. However it was observed that, even though cardamom is considered to be shade loving under

growth plant, the entry of *Njallani* has changed the scenario. *Njallani* needs more sunlight for its growth and its cardamom production largely depends on the availability of direct sunlight. This has led to pruning and lopping of forest canopy on regular basis. *Njallani* was introduced to the region during mid - 1990's. The high capsule production by this variety has a major role in the increased production of cardamom from CHR hills during the past two decades. Along with the varietal change, increased use of chemical agents such as fertilizers and pesticides for better crop protection and yield has also contributed to this change.

Cardamom used to be a shade loving plant seen as undergrowth in the tropical rain forests of India, now no more remains in the same nature. The typical tropical rain forest ecosystem consists of highest level of biodiversity comprises of thousands of plants, insects and many species of vertebrates. However the cardamom monoculture has changed the ecosystem drastically and led to a homogenous system. The varied agricultural practices were revealed through the intensive survey we carried out in the region. A thorough cleaning of the farming land, severe pruning and shade lopping of tree cover and removal of under growth are the main reasons for the loss of biodiversity of the region. Regeneration of the pruned trees seems to be very slow in the region, this could be because of local climatic and environmental factors and alteration in the soil fertility due to increased application of chemical fertilizers.

4.1.3. Pest incidence and diseases of cardamom

All the farmers interviewed were skeptical on the pest incidence in their cardamom plantations. The major pets reported are, *Hilarographa caminodes* (root borer), *Dichocrocis (Conogethes) punctiferalis* (shoot borer) and *Sciothrips cardamomi* (thrips). Other pests like capsule borers (*Jamides* sp), cardamom aphid (*Pentalonia nigronervosa f.Caladii*), beetle borer (*Onthophgus* spp), root grubs (*Basilepta fulvicorne*), thrips (*Scothrips Cardomomi*) and hairy caterpillars (*Eupterote cardamom*) are also reported. Since the pest causing damage is disastrous in most of the cases, the farmers apply the pesticides in a prophylactic mode without waiting for the pest outbreak to happen. Number of insecticide sprays ranged from 6-24 times/year for fungicides and 2-4 times/year for nematicides. The number of insecticide sprays given against thrips and borers ranged from 12-14 times/year.

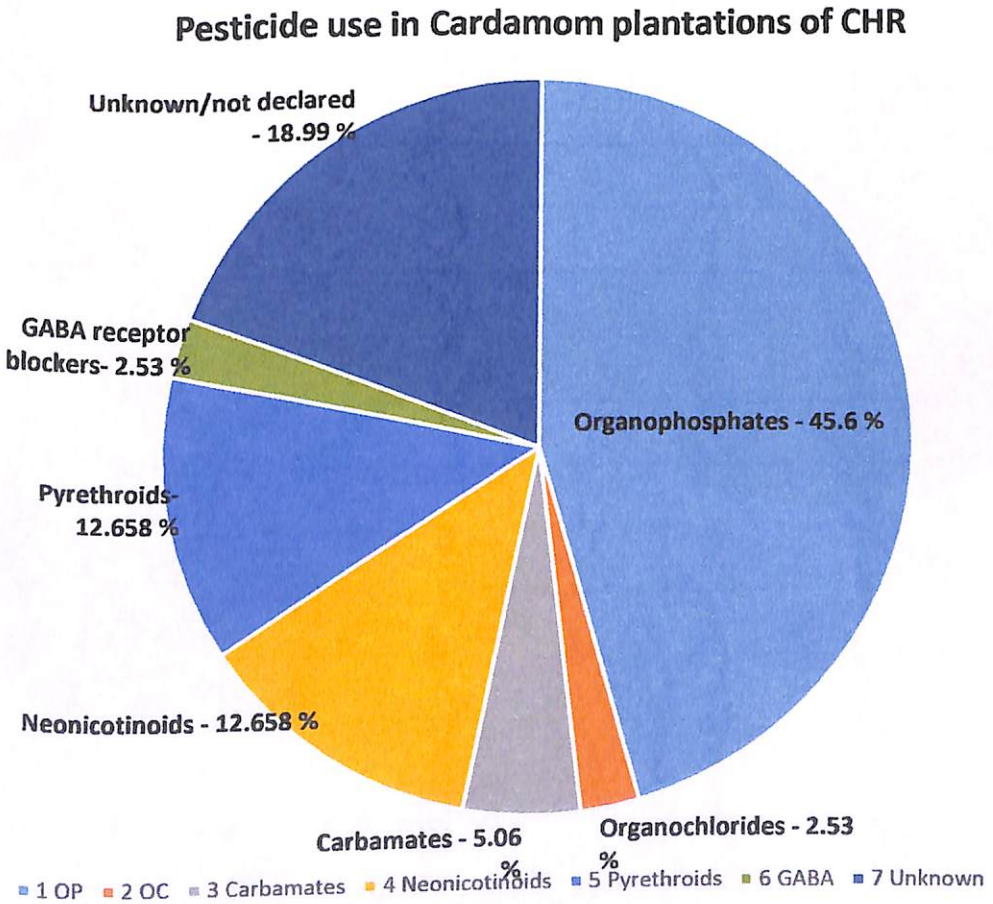
4.1.4. Major pesticides used in cardamom plantations of CHR

The interactions with big farm agriculturalists, house hold farmers, casual labourers, pesticide shop owners, Govt. officials - revenue, agriculture and health authorities etc., has provided ideas on the extent of pesticide use in the region. Most of the organochloride pesticides are banned by government and are not

available in the market, instead other group of chemicals are recommended. A detailed list of recently banned chemicals and their substitutes are given in Kerala Government order "GO (M.S) No 116/2011/Agriculture dated 07/05/2011".

Interactions with farmers were helpful in understanding the pattern of use of pesticides and agricultural practices. Irrespective of the pest attack, some or other pesticides are applied in every 15 days. For the application of fertilizers or pesticides, the farmers depends on fertilizers/pesticides manufacturer's representatives or suppliers. More studies and interactions are required to identify the exact nature of the farm practices followed in the small holdings and big estates. Based the survey conducted in the region, the chemical nature the pesticide use can be classified as organophosphates – 45.6 %, pyrethroids – 12.658 %, neonecotinoids – 12.658 %, carbamates – 5.06 %, GABA receptor blockers – 2.53 % and organochlorides – 2.53 %. The study identified that 18.99 % of the pesticides were not identified properly by the users (Figure 1.2). Residues of chlorpyrophos, quinalphos, dimethoate and arsenous acid were detected from soil samples collected from cardamom plantations located in different parts of CHR

Figure –1.2 : Percentage contribution of pesticide use in plantations of CHR



4.1.5. Qualitative analysis of pesticide residues in different matrices

The pesticide residues in 384 samples from 96 locations spread across Cardamom Hill Reserves were analysed. The residues were identified from 46 samples from 16 locations. Chlorpyrifos, quinalphos and arsenorus acid were the pesticide residues identified from the region. Among 46 samples identified with the presence of residues, 45 samples belongs to cardamom cultivation areas and one sample with the presence of arsenous acid was from a mixed cultivation area with cocoa, cardamom, pepper and coffee. Among the samples identified, chlorpyrifos alone was present in 18 samples, quinalphos alone was present in 12 samples, arsenous acid alone was present in 06 samples and 10 samples showed the presence of both chlorpyrifos and quinalphos residues.

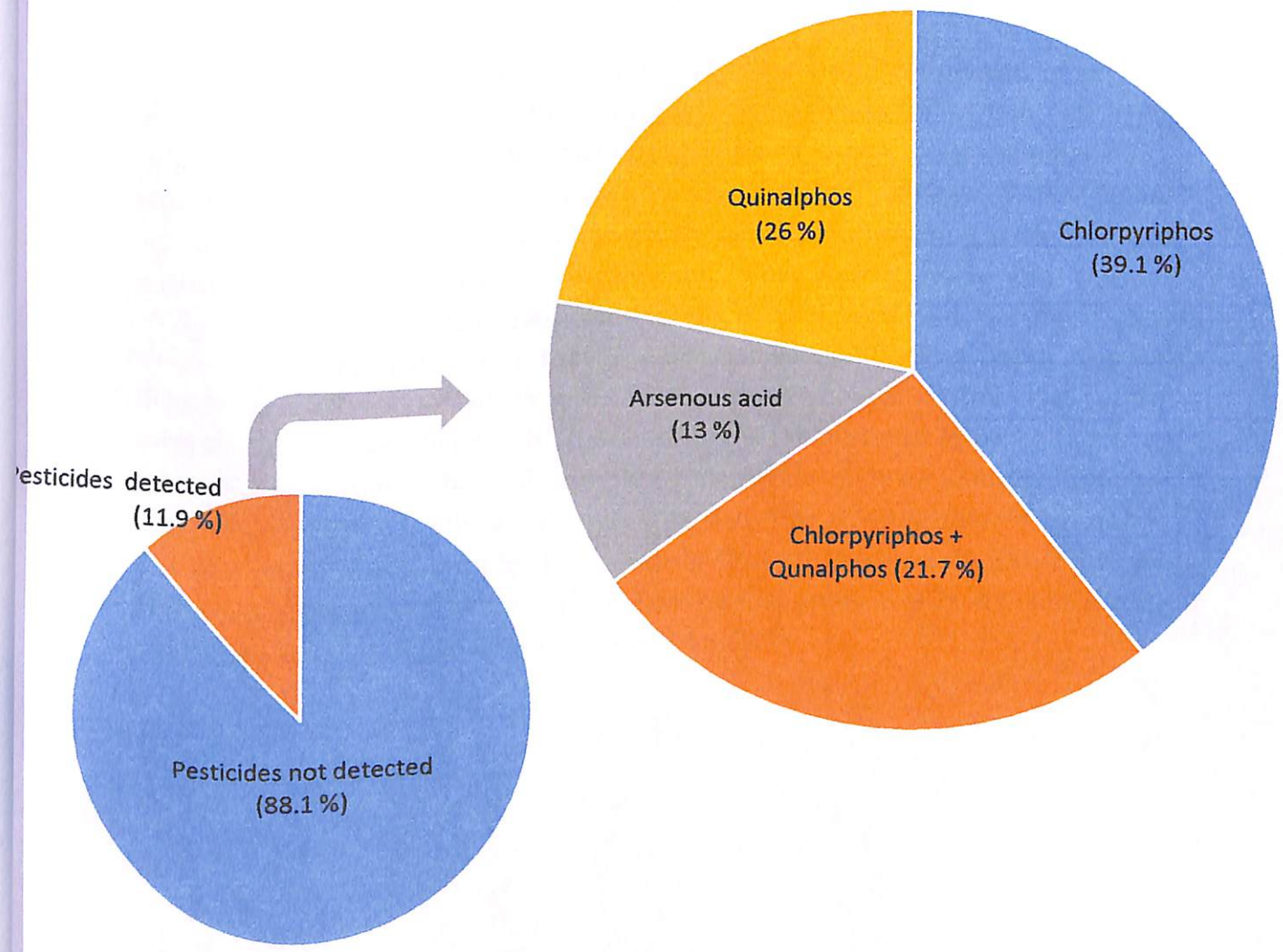


Figure 1.3 : Pesticide residues detected from CHR soils

Pesticide residue analysis of the soils collected from the region were carried out using gas chromatograph mass spectrometer following standard procedures. As specified in the materials and methods, the total area of the region was divided in to grids of 6.25 km^2, from these grids samples were collected from the 7^{th} grid following randomized sampling method. A total of 384 samples from 24 grids (4 replicates from four locations in each grid) were analysed. Among this 46 samples (11.97 %) were identified with the presence of different pesticides. Presence of chlorpyrifos, quinalphos and arsenous acid were found from the soils. Among samples identified with pesticides, 39.1 % samples had the presence of chlorpyrifos, 26 % had quinalphos, 21.7 % had the presence of both chlorpyrifos and quinalphos and 13 % samples showed the presence of arsenous acid (Figure-1.3).

4.2. Soil microbial dehydrogenase activity

Soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and play crucial roles in carbon, nitrogen, sulphur and phosphorus cycles. Dehydrogenases are respiratory chain enzymes, which plays major role in the energy production of organisms. Dehyrogenase activity can therefore be used as an indicator of biological redox systems and as a measure of biological activity in the soil. The present study has showed considerable soil dehydrogenase activity in control soils. All the three plots showed significantly different dehydrogenase activity at 0-20 cm depth. The soil dehydrogenase assay of all 23 grids were compared with control. The control samples showed significantly high dehydrogenase activity in terms of triphenyl formazan formation. The soil samples from grids showed entirely different spectrum of activity levels and falls in to two groups when compared to control. The first group of soil samples from 9 grids showed significantly lower activity. The same grids were identified with presence of pesticides in the soil during qualitative analysis of pesticides (Figure -1.4 A & B)

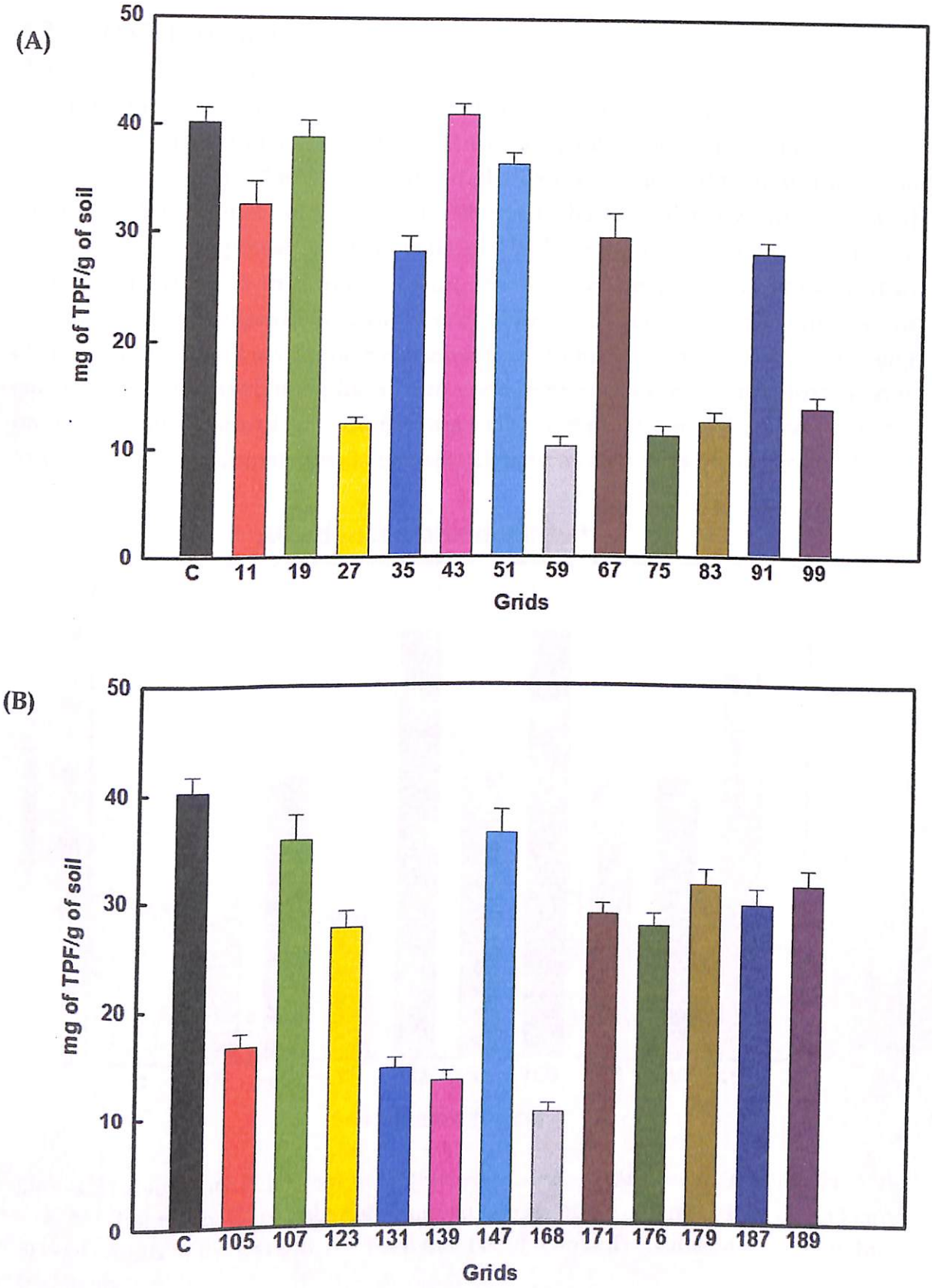


Figure 1.4 A & B : Microbial dehydrogenase activity in soils of Cardamom Hill Reserves. The results were expressed as the formation of triphenyl formazan. The values are mean ± S.E. of three experiments.

4.3 Toxicity studies

4.3.1 Avoidance behavior test

The preliminary results of the avoidance behaviour test indicated that 100% of the organisms were found in the control soil samples (soil without any contamination), indicating that the contaminated soil samples can be considered toxic and showing limited habitat function. The study showed that the earthworms exhibited significant net response towards soils of CHR on exposure. The net response varied from 20-60 % in different grids. However no response was observed in control soils. Earthworms can sense chemicals *via.* a large number of chemoreceptors, and avoid the contaminations. In the present study, we observed earthworms that were introduced into containers containing contaminated soils on one side and control soils on the other side, the preference of the earthworms towards non-contaminated soils were significant as shown in the Figure – 1.5.

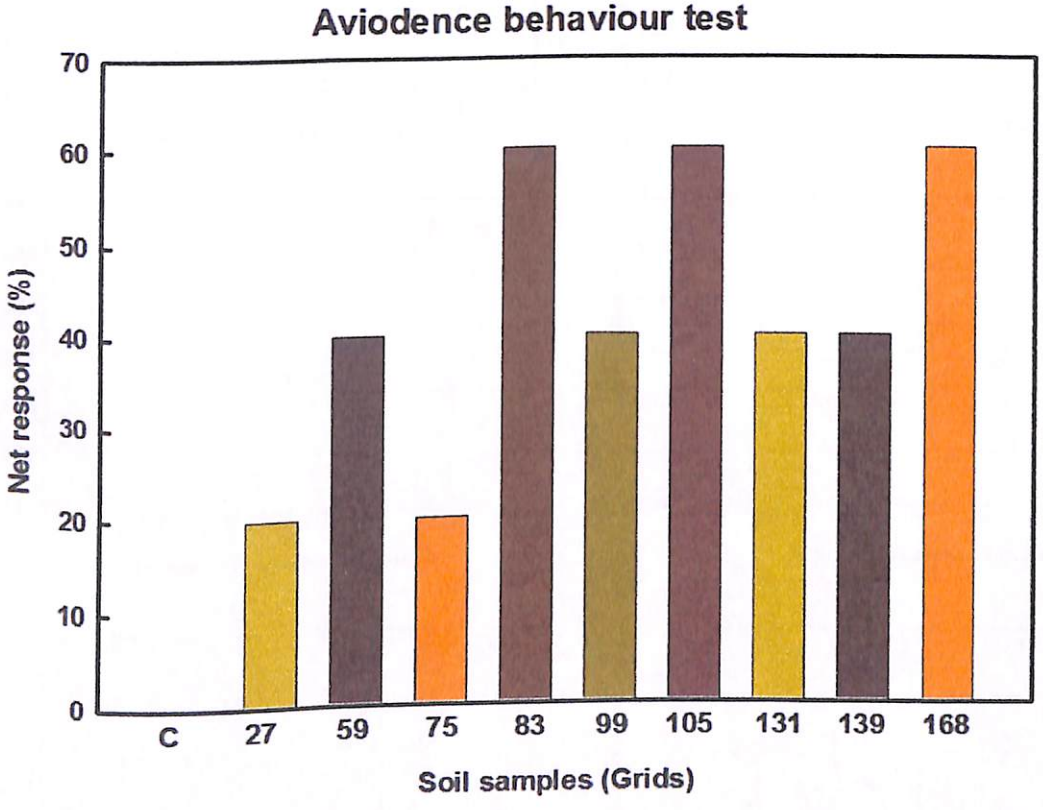


Figure -1.5 : Avoidance behaviour of earthworms *Eisenia fetida* towards soils of CHR. NR = C-T/N x 100 where C- Number of observed worms in the control soil, T - Number of observed worms in the test soil, N - Total number of worms The values are mean of three experiments.

4.3.2 Weight loss and mortality

Weight loss is a typical symptom of toxicity effect irrespective of model organisms and exposed toxicants. In the present study of chlorpyrifos exposure, a

dose and exposure time dependent weight loss was observed in exposed earthworms as shown in Figure-1.6. Observations were made on 0, 1, 3, 7 and 14 days respectively after the exposure of the pesticide. Control group does not show any significant change in the body weight till 14 days. Lower dose of chlorpyrifos (10 mg/kg) also showed a similar trend. All the other doses (20, 40, 80 & 100 mg/kg) showed weight loss with increase in exposure time and found to be significant from 3^{ed} day of exposure on wards.

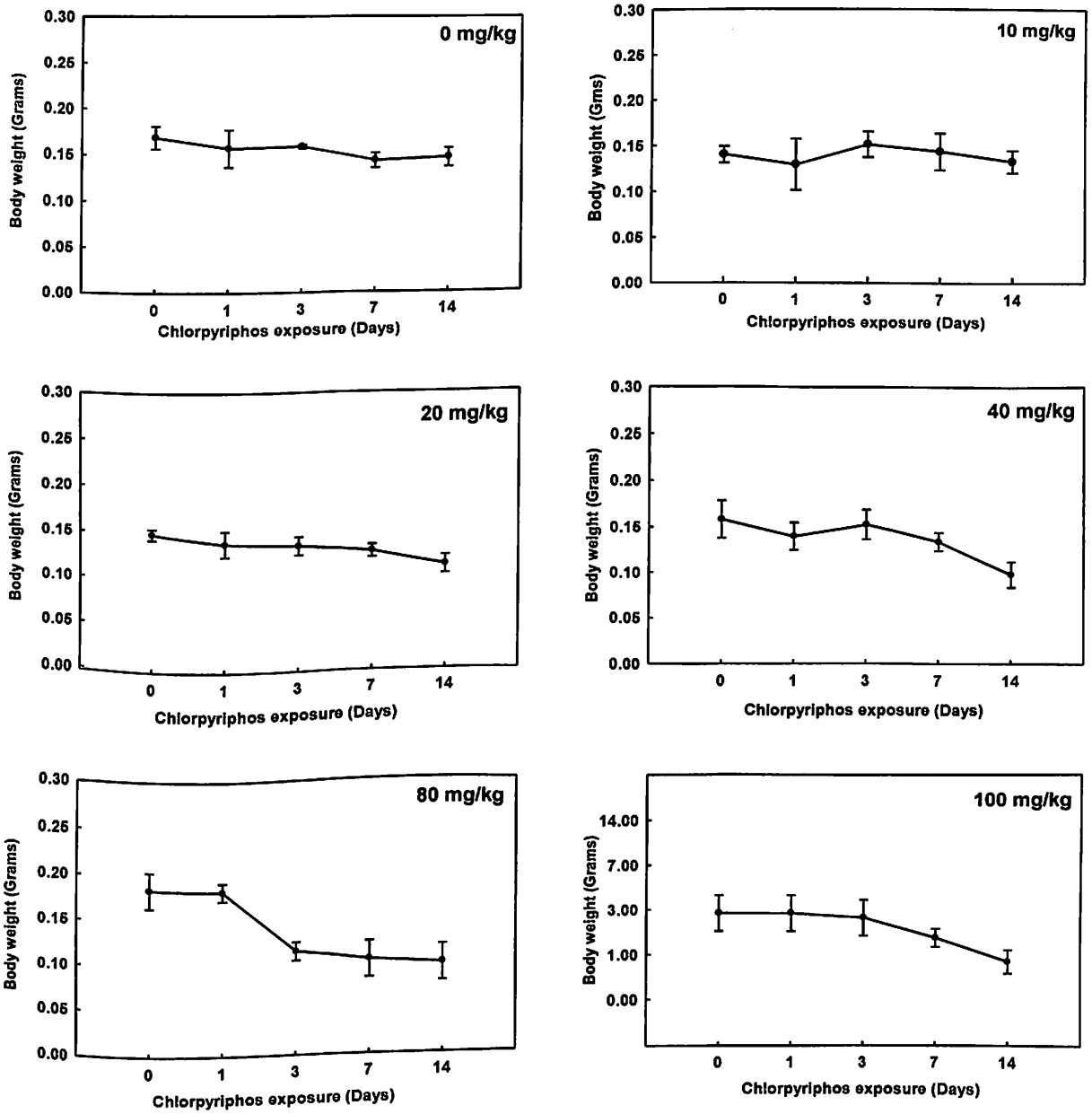


Figure - 1.6 : Effect of chlorpyrifos exposure (0, 10, 20, 40, 80 & 100 mg/kg) on weight of earthworms *Eisenia fetida*. The values are mean ± S.E. of three experiments. * Significantly different from control at $p \leq 0.05$ by Dunnet's test.

4.3.3 Biochemical variables

Effect of chlorpyrifos exposure on lipid peroxidation levels in earthworms

On 14th day of the chlorpyrifos exposure, there was a drastic increase in the lipid peroxidation levels as shown in the results. The chlorpyrifos exposed groups showed a significantly high lipid peroxidation levels compared to control. The initial dose of chlorpyrifos (10 mg/kg), resulted a 3 fold increase in lipid peroxidation. The higher doses of exposure showed further significantly higher values compared to control. However there was no significant difference among the pesticide treated groups (Figure - 1.7).

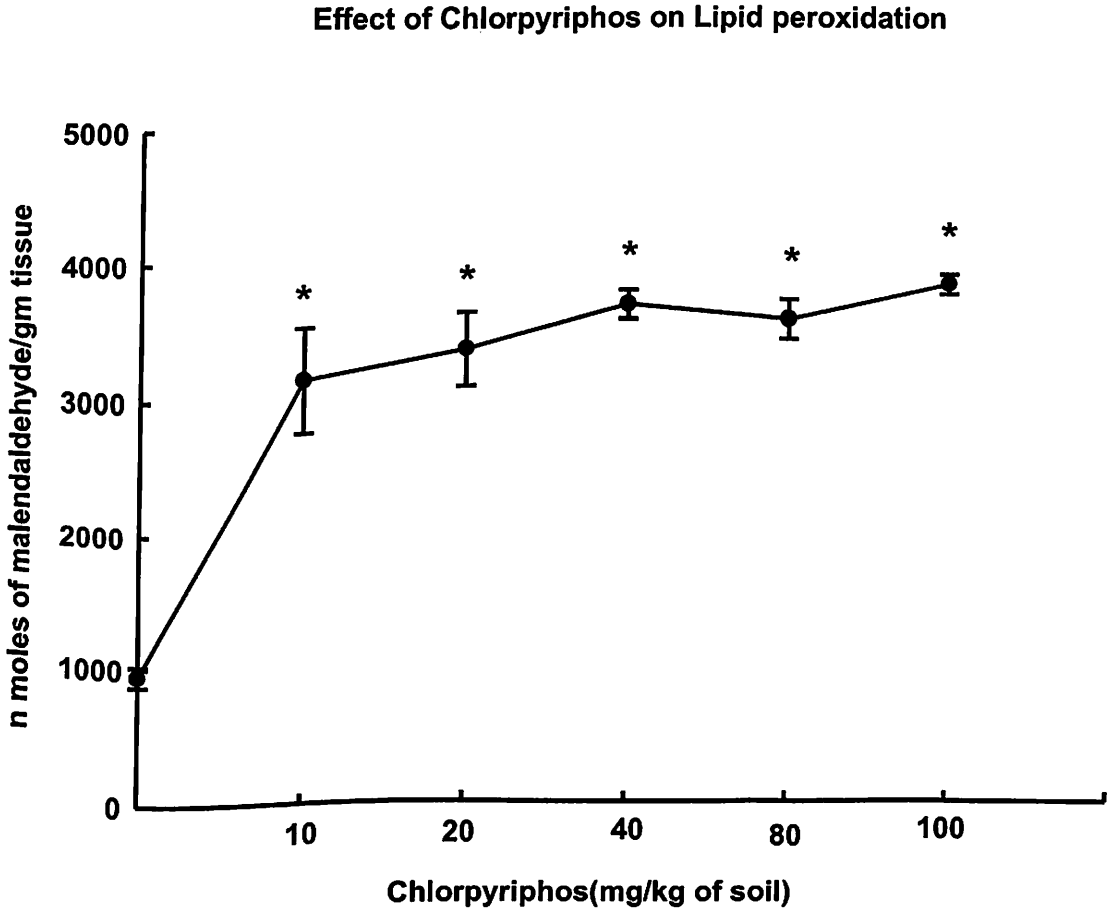


Figure - 1.7 : Effect of chlorpyrifos exposure (0, 10, 20, 40, 80 & 100 mg/kg) on lipid peroxidation levels on earthworms *Eisenia fetida*. The values are mean ± S.E. of three experiments. * Significantly different from control at $p \leq 0.05$ by Dunnet's test.

Effect of chlorpyrifos exposure on Total protein content in earthworms

A significant decrease in total protein content was observed in earthworms of 14th day chlorpyrifos exposure of high doses (80 and 100 mg/kg). Decrease in

metabolism and protein synthesis are the foremost manifestations of pesticide toxicity. A similar trend was observed here too. The initial doses (10, 20 and 40 mg/kg) does not show any significant difference from control (Figure - 1.8).

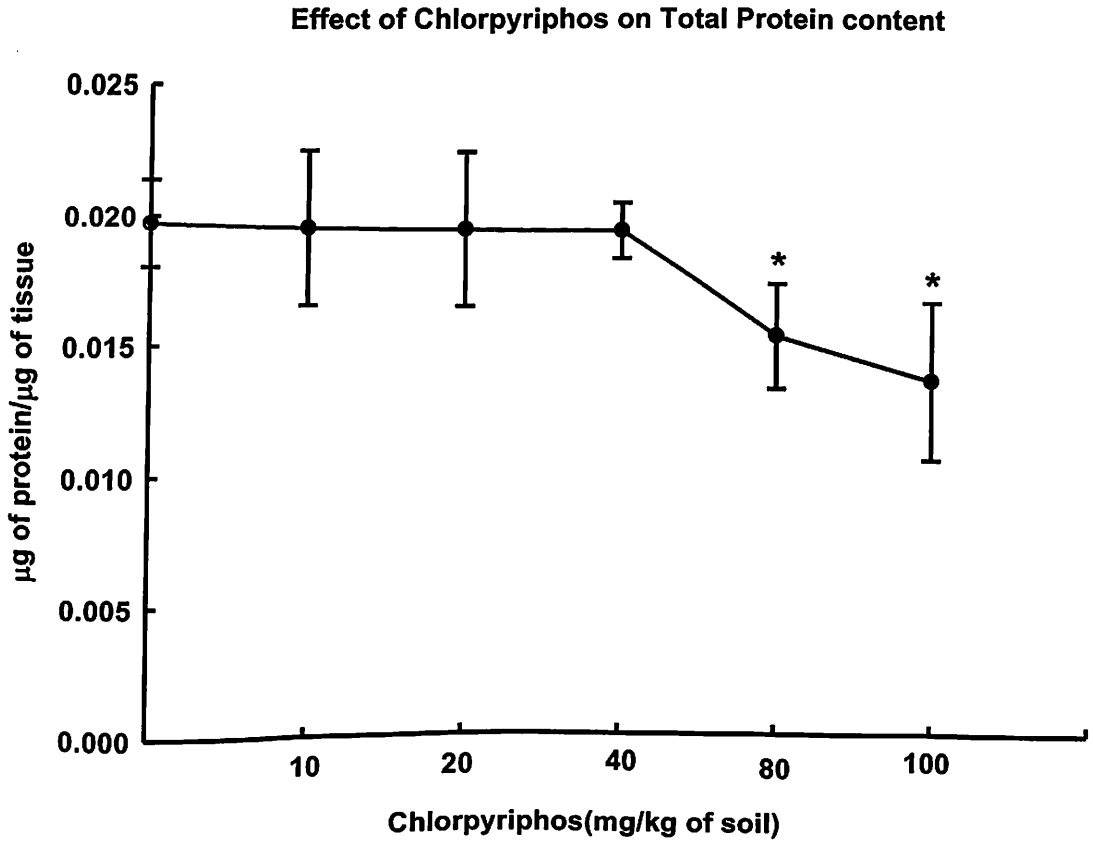


Figure - 1.8 : Effect of chlorpyrifos exposure (0, 10, 20, 40, 80 & 100 mg/kg) on total protein content in earthworms *Eisenia fetida*. The values are mean ± S.E. of three experiments. * Significantly different from control at $p \leq 0.05$ by Dunnet's test.

4.3.4 Histopathology

The effects of chlorpyrifos on the histology of the earthworm *E. fetida* after 14 days exposure are shown in Figure - 1A-F. The control animals showed normal cellular structure. Histologically integument of earthworm *E. fetida* is differentiated into cuticle, epidermis, (a single layer of cells) and a double layer of muscle fibers. The body wall is covered externally by a thin, pervious and flexible cuticle. The cuticle is supported by the underlying epidermis. A dose dependent alteration in the cellular structure was observed in exposed animals. Continuous exposure of chlorpyrifos leads to irreversible toxicity effects with protein degradation, cell

membrane damage, cellular infiltration and inflammation as shown in figures (Figure 1.9 A-D)

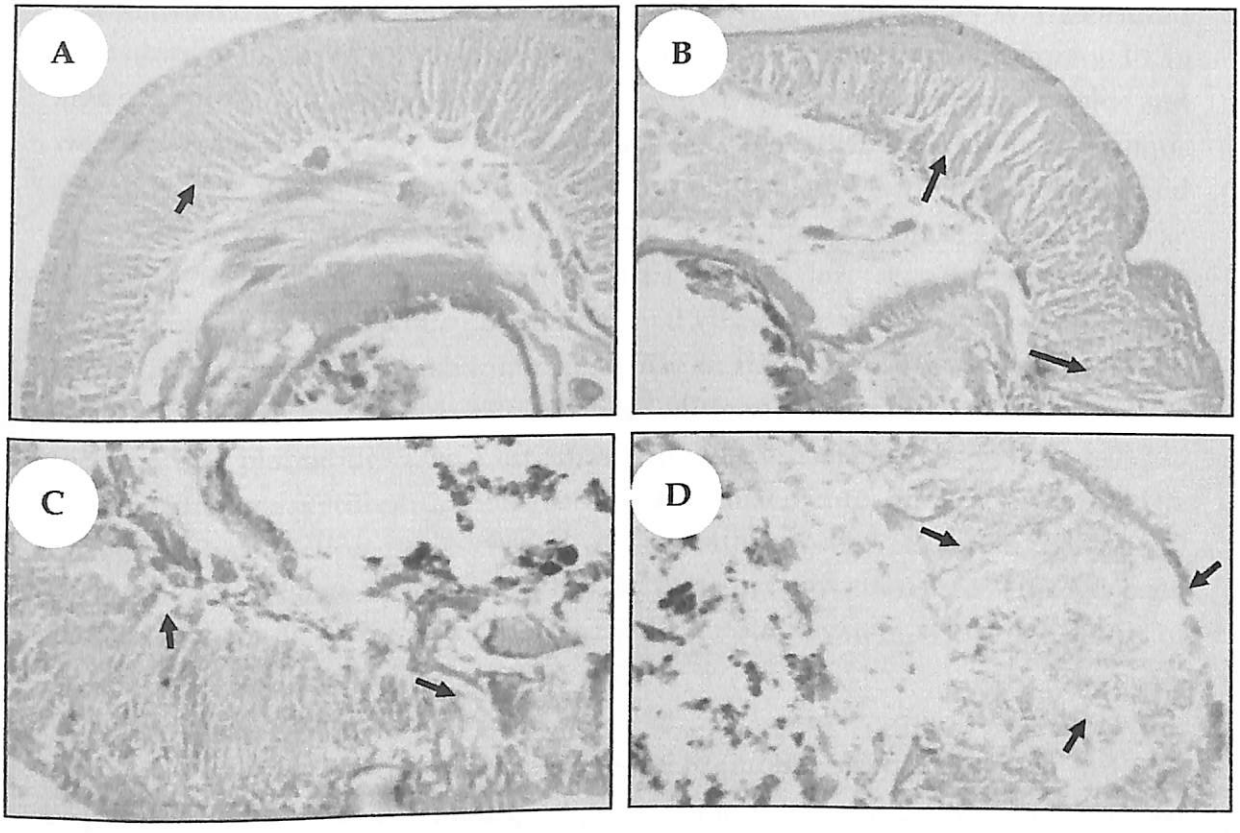


Figure – 1.9 : Effect of chlorpyrifos exposure on histology in earthworms *Eisenia fetida*. (A) Control, (B) 10 mg/kg, (C) 80 mg/kg, and (D) 100 mg/kg

5. Discussion

The Cardamom Hill Reserve (CHR) of Southern Western Ghats is a treasured repository of biodiversity of spices. It contain wet evergreen forests spanning to an area of about 86,000 hectares. A comparatively diverse physiographic, edaphic and weather condition of the Western Ghats perhaps attributed to its enormous diversity of many endemic plant species, agricultural and horticultural crops and their wild forms. Cardamom Hill Reserves, as the name implies, houses the best locations for cardamom cultivation. This region has the finest cardamom plantations in India. Because of the optimal climatic conditions, the cardamom plantations occupies the maximum land use in the region. However due to the fluctuation in the cardamom price and high cultivation costs, there has been a slow shift to other plantations also. Our survey in the study region showed that 78.125 % of the land use is still cardamom, followed by mixed crop, tapioca, cocca etc. The cardamom production has recorded a maximum growth rate from 200 to 800 kg/ha, over last 20 years in spite of the decrease in cultivation area. This has been achieved through an intensively managed cropping system with high input of agricultural chemicals and fertilizers.

Presently "Njallani" is the most preferred cardamom variety cultivated in CHR. Cardamom being a shade loving crop, is planted as undergrowth in the forest regions. However with the entry of Njallani, a variety needs more sunlight for their optimal growth, the scenario has changed. This led to the pruning and lopping of forest canopy on regular basis. Such activity had a severe impact on the ecosystem and microclimate of the region. A substantial negative impact of shade removal on the microclimate of cardamom has been noticed and the influence is typically echoed on soil temperature and moisture regimes. Also there has been reports on the non-influence of shade lopping on photosynthetic efficiency of cardamom plants.

Canopy lopping is done on the assumption that lopping can enhance cardamom yields through increased photosynthetic activity of cardamom plants. However, photosynthesis is observed to be maximum in cardamom plants during low-light monsoon period (July and August) even under severe lopping situations also. The photosynthesis is observed to be higher when soil moisture and nutrients are also high. Hence severe shade lopping for the purpose of increasing photosynthesis has found to be of no use, adversely it is leading to the degradation of rainforest structure and function (Miniraj and Murugan, 2000). Other than the loss of biodiversity and ecological niche, severe lopping may lead to change in microclimatic conditions too. One of the major negative impact of such change is the insurgence of pests and insects. Both useful and damaging insects and pests

will respond to warmer environments, and an overall upsurge in insect abundance is expected in mid-latitudes and high altitude tropics. But the main effect could be on the changes in the survival, generation frequency and numbers and distribution boundaries (Fuhrer, 2003)

The pest incidence in the cardamom plantations is a common issue faced by farmers. The change in climatic conditions and frequent occurrence of break periods during monsoon months, with raised soil temperature, favored soil insect pests like root grubs and plant parasitic nematodes along with white flies and thrips (Murugan, 2011). Since the pest attack is an unpredictable one leading to total destruction of the crop, the pesticide applications are many times prophylactic in nature. It has been noted that the insecticide sprays ranges from 12- 14 times per year. This seems to be a high use and such a chemical dose may adversely affect the ecosystem. Among the surveyed region, the major class of pesticides used are organophosphates followed by neonicotinoids, pyrithroids, carbomates, organochlorides and GABA receptors. Among the respondents to the survey, 18.99% were unwilling to provide details of their pesticide use.

Soil samples collected from 96 locations in the designated study area were subjected to pesticide residue analysis and 11.9 % of samples has shown the presence of chlorpyrifos, quinalphos and arsenous acid. It has already been established in the survey that organophosphates are the major classes of pesticides used in the region. However residues could be detected in only few samples. There could be many reasons for the non-detection of pesticides in soil samples from the region. The major reasons could be i) degradation of pesticides and ii) leaching of the pesticides during rains and irrigation.

Earlier the organochloride (OC) class of chemicals are one of the major group of agents which were in use against most of the pests of cardamom plants. However due to the high half-life, concerns over the toxicity and adverse health issues and pressure from the environmental safety groups has led to the replacement of most of the organochloride class of pesticides with organophosphates and other ecofriendly chemicals. Even though the organophosphates are also reported with safety issues, the degradation time is relatively much faster than that of OC compounds.

Soil flora plays crucial role in the maintenance of soil conditions. Microbial dehydrogenase activity in the soil has been shown to be associated with other measures of microbial activity such as proteolytic activity, nitrification potential and carbon dioxide release. It is also related to the level of total organic matter and

to the activities of soil enzymes such as phosphatase. We have analyzed the dehydrogenase activity from soils of all the grids. Activity is usually highest in the top few centimeters of soil, and therefore, the depth at which the soil is sampled can have a significant effect on the observed activity of the soil. The samples collected from 0-20 cm of the soil showed significantly varying levels of activity. Control samples were drawn from an undisturbed area the same agro eco-geographical conditions. Out of the 24 samples analyzed, 09 samples showed considerably lower dehydrogenase activity. This is well correlated with the qualitative detection of pesticides from the samples. The dehydrogenase test is performed under controlled laboratory conditions, and actual activity in the field may vary with changes in soil temperature, pH and moisture content. However a similar correlation may be expected from *in situ* conditions also.

To evaluate soil quality, bioassays can be valuable tools to measure the possible toxicity of contaminants focusing on their bioavailable fraction. Earthworm avoidance test was one of the methods developed for finding out the favorable environment of survival of terrestrial organisms. The acute earthworm avoidance test was first developed in 1996 (Yeardley et al., 1996). International Standards Organization (ISO) had further established it as test for soil functions and developed as a method for rapid screening and evaluation of soil function and influence of contaminants and chemicals on earthworm behavior (ISO, 2008). These tests were accepted across the globe for screening of contaminated soil and soil functions (Environment Canada, 2004; Schaefer, 2004; Yeardley et al., 1996). Earthworms were opted as test-organisms in these tests because they are common in a wide range of soils, representing 60-80% of the total soil animal/invertebrate biomass. In the present study, the experiment was conducted with soils from the grids identified with presence of pesticides and earthworms has shown considerable avoidance to these soils compared to control soils. The analysis of soils from CHR study area has exhibited significant net response from earthworms which varied from 20-60 % in different grids. The preference of the earthworms towards non-contaminated soils were significant as shown in the results. Similar results has been reported before also. According to the results of Hund-Rinke & Wiechering (2001), the avoidance behaviour test with samples of contaminated areas showed a significantly larger sensibility compared to those with artificially contaminated samples. Earthworms exposed to carbendazim and benomyl showed the same pattern, avoiding the soil at concentrations equal or higher than 10 mg/kg of soil (Loureiro et al., 2005).

To study the sub-lethal toxicity of the mainly used organophosphate pesticides used in the region, chlorpyrifos is taken as a candidate molecule for further toxicity

studies. Even though avoidance behaviour tests being used as chronic tests in assessing a sublethal toxicity, mortality can also be considered an important evaluation endpoint in these tests. In some chemical doses, earthworms were found dead in the control portion, this could be because of some of these chemical compounds affect the nervous system and therefore will disorient the earthworms which leads to their inability to escape. Organophosphate class of compounds are reported to affect the earthworm behaviour. Dimethoate, one of the major organophosphate is a potent inhibitor of acetylcholinesterase, and is found to influence earthworms behaviour (Martikainen, 1996; Ribeiro et al., 1999). Also the biomass change in earthworms were reported up on pesticide exposure. The present study also reported similar results with significant biomass loss at higher doses of chlorpyrifos exposure. Martikainen (1996) observed IC50 values for biomass change in the range of 14.4 to 42.9 mg /kg of soil for the earthworm *Aporrectodea caliginosa tuberculata* in case of dimethoate exposure.

Alterations in biochemical variables are always indicators of the extent of toxicity to the living systems. The present study has evaluated lipid peroxidation, total protein and histological parameters as markers of chlorpyrifos toxicity in earthworms. Lipid peroxidation levels has shown a significant elevation with dose of the pesticide. The lowest dose of chlorpyrifos (10 mg/kg) itself had shown a significant lipid peroxidation which was further elevated along with the increase in dose of exposure. Not much variation was observed among the doses of exposure.

In addition to the total biomass, total protein is another indicator, we analyzed for accessing the toxicity of chlorpyrifos. A dose dependent decrease in the total protein levels were observed with increase in the exposure of pesticide which was significant from 80 mg/kg. Growth inhibition can be a good indicator of chemical stress, chemical effects that can link to the dynamic energy and ultimately inhibit the growth of the tested organisms. The slight decrease in weight of earthworm in control oils suggested that soil nutrients were sufficient to maintain the survival of earthworms, but insufficient to allow further growth and loss of protein is an indicator of the toxicity levels. The results in growth inhibition treated by the two pesticides agreed with those reported in other pesticides. A dose dependent decrease in the growth of *E. fetida* exposed to dieldrin at several sub-lethal concentrations was reported (Shi et al., 2007). Growth inhibition in terms of biomass loss could be an indicator of protein loss.

Alterations in internal organs as observed in histological analysis could be a direct evidence to the damage caused by the toxicity on exposure to chlorpyrifos.

Histologically the natural covering of earthworm *E. fetida* is differentiated into cuticle, epidermis, (a single layer of cells) and a double layer of muscle fibers. The body wall is covered externally by a thin, pervious and flexible cuticle. The cuticle is supported by the underlying epidermis. A dose dependent alteration in the cellular structure was observed in chlorpyrifos exposed animals. Continuous exposure of chlorpyrifos leads to irreversible toxicity effects with protein degradation, cell membrane damage, cellular infiltration and inflammation. Major histopathological effects included slight vacoulations of the epithelium particularly the proximal areas. Butachlor exposed *Perionyx sansibaricus* has shown altered histopathology in the form of destruction of the peritoneum (chloragogenous layer) and epithelium (Gobi et al., 2005). Carbaryl exposed *Pheretima posthumus* had shown damage to the chloragogenous layer cell (Gupta and Sundaraman, 1988). Glyphosate is reported to cause damage to the epithelial tissues of *Pheretima elongate* (Mohssen, 2000). A range of concentrations of benomyl (8.3, 56, 112 mg/kg) used in a spermatogenesis study caused abnormalities in the ultrastructure of sexual organs of *E. fetida* (Sorour and Larink, 2001). Major observations were, loss of mascular compactness, nuclei loss in epithelial cells and disruption of natural muscular architecture. Besides being damaged, the epithelial tissues had prominently folds with glandular enlargements. These finding were in tune with earlier reports on the histological alterations caused by pesticides in earthworms. In nutshell, the pesticide use has diverse effect on the terrestrial organisms and microbial diversity of the region.

Section -2

Effect of Pesticide application on the photosynthetic efficiency and karyotype abnormalities in dominant shade tree species in Cardamom Hill Reserves

1. Introduction

Cardamom Hill Reserves (CHR) or 'cardamom hills' consists of mid elevation tropical evergreen forest located in the South Western Ghats of Kerala, India. For the past century cardamom is cultivated as an under storey crop in the CHR of South Western Ghats. Tropical evergreen forests of Kerala are considered as the original home of small cardamom (*Elettaria cardamomum* (Sivanadan *et al.*, 1986). Cardamom grows luxuriously under the forest tree canopy of South Western Ghats. CHR contributes a major share in the total production of cardamom in India (Murugan *et al.*, 2007). Intensive cultivation of cardamom is however adversely affecting the forest tree community in CHR. Excess use of pesticides and chemical fertilizers for enhancing the production of cardamom will adversely affect the forest ecosystem in many aspects. This study is mainly aimed to trace out the effect of pesticides in plant community by measuring the photosynthetic efficiency and karyotype abnormalities in dominant shade tree species in CHR.

2. Objectives

- Karyotype analysis of dominant and keystone tree species in cardamom cultivation area of CHR.
- Measure the photosynthetic efficiency of dominant and keystone tree species in the cardamom cultivation area of CHR.

3. Materials and Methods

3.1. Sample collection

The samples for the study were collected from the designated study area (Figure 3.1) as briefed earlier section.

3.2. Karyotype analysis of dominant trees in CHR

3.2.1. Identification of dominant trees in cardamom cultivation area of CHR

Transect method was used to quantify the woody vegetation above 10.0 cm GBH. Twenty two quadrats of size 50 m x 50 m were laid along the transect in an elevation gradient ranging from 800 to 1400 m mean sea level (MSL). Eleven plots were established in the altitude range of 800-1100 m MSL and remaining eleven plots in the altitude range of 1100 -1400 m MSL (Fig 1). The total area sampled in this study is 5.5 ha. All woody vegetation above 10 cm GBH at 1.37 m height was enumerated and tagged. GBH was measured on the side of the tree facing the slope. The vegetation data were analysed for relative density, relative frequency, relative

dominance (Phillips, 1959; Kershaw, 1973) and the sum of values for these parameters represented by Importance Value Index (IVI) for different species (Curtis, 1959).

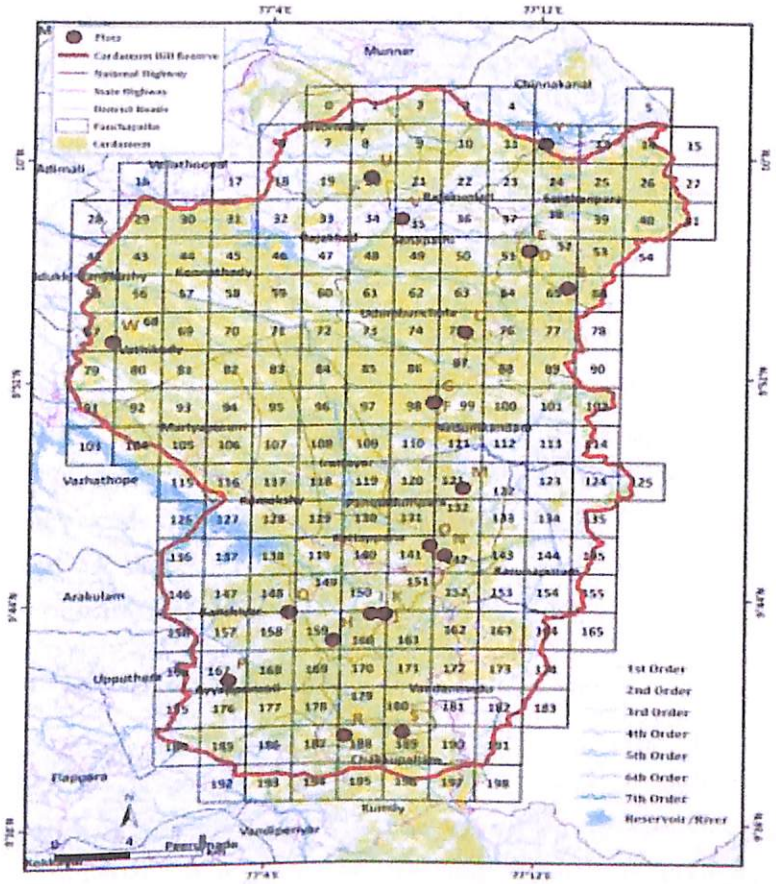


Figure 2.1 : Map of Cardamom Hill Reserve showing locations of the sample collection area. Samples were collected from the locations marked with dots

3.2.2. Nursery establishment of dominant trees

Seeds of dominant trees species were collected during the season from the area where cardamom is intensively cultivated. Collected seeds were washed in tap water and soaked in water overnight. Water soaked seeds were sown in plastic tray filled with vermiculate and kept in controlled condition. Germinated seedlings were carefully collected from the tray and bagged in polythene bags filled with potting mixture (1:1:1). Bagged seedlings were kept in shade and irrigated regularly.

3.2.3. Karyotype analysis

Root tips of six month old seedlings were collected for the purpose of karyotyping. Root tips were collected in the late afternoon hours and washed in tap water for five minutes. Root tips were collected using a surgical blade and were pretreated with saturated solution of α-bromo naphthalene for 16 h at 50^{0}C. Pretreated root tips were fixed with 90 percentage of acetic acid for 30 minutes at room temperature. Fixed root tips were then washed in 95 % ethanol for five minutes and stored in 70 % ethanol. The stored root tips were used for the study.

The stored root tips were washed three times in tap water. Each washing took five minutes. Washed root tips were hydrolyzed with 1N HCl for ten minutes at 60^{0}C. Hydrolyzed root tips were macerated with two percentage of pectinase for forty minutes at room temperature and stained with Schiff's reagent for one hour at room temperature. The stained root tips were washed with distilled water for ten minutes, the meristematic zone was isolated and squashed between the slide and the cover slip in a drop of 1% Belling's acetocarmine. Then the prepared slide was observed in 100x objective of a microscope (Jahier et al, 1992).

3.3. Photosynthetic efficiency test

Photosynthetic efficiency test was done to identify whether the seedlings were in environmental stress or not. The plants used for the study were kept under controlled condition. The photosynthetic efficiency of the seedlings under controlled condition was measured using Photosynthetic Efficiency Analyzer (PEA, Hanzatech, U.K.). The PEA measurements were conducted in the morning hours (6.30 am to 7.30 am). Mature healthy leaves of the seedlings were capped using leaf cap for twenty minutes. After twenty minutes the sensor head of the PEA was connected to the leaf cap gently, the shutter of the leaf cap was removed and the remote trigger button was pressed. After completing the measurement, the reading was taken and saved.

An experimental trial was also conducted under the controlled condition to find out whether application of pesticide generates any stress on seedlings. Two species such viz., *Artocarpus heterophyllus* and *Cullenia exarillata* were used for the purpose. Three different dosages ; pesticide with normal dosage (Chlorpyrifos 20 EC 0.04 per cent) half the recommended dosage (Chlorpyrifos 20 EC 0.02 %) and double the recommended dosage (Chlorpyrifos 20 EC 0.08 %) of pesticide and control were used in this study. Pesticide was applied in one month interval for a period of three

months. Photosynthetic efficiency of seedlings was noted before and after the application of pesticides. Data was collected at 20 days interval.

4. Results

4.1. Karyotype analysis of dominant trees in CHR

4.1.1. Identification of dominant trees in the cardamom cultivation area of CHR

From the present study 1737 stems were recorded from the selected plots of CHR, with an average stem density of 316 stems/ha with a maximum of 788 stems/ha and minimum of 168 stems/ha. A total of 99 tree species were recorded from 22 transects of 0.25 ha representing 76 genera, 35 families and two sub families. Six species were only identified up to the genus (Table - 2.1).

Table - 2.1: Importance Value Index of the tree species.

Species	No. Ind	RD	RF	R. Dom	IVI
Vernonia arborea	410	23.604	5.556	8.405	37.565
Artocarpus heterophyllus	344	19.804	5.556	8.699	34.059
Toona ciliata	160	9.211	5.833	8.431	23.475
Persea macrantha	103	5.93	4.722	8.991	19.643
Dimocarpus longan	37	2.13	3.889	5.646	11.665
Bischofia javanica	24	1.382	3.333	5.905	10.62
Erythrina subumbrans	94	5.412	2.5	1.881	9.793
Elaeocarpus tuberculatus	30	1.727	3.056	4.566	9.349
Canarium strictum	28	1.612	3.056	2.943	7.611
Cullenia exarillata	20	1.151	1.111	5.209	7.471
Myristica beddomei	18	1.036	1.944	3.472	6.452
Ficus beddomei	18	1.036	2.778	2.268	6.082
Palaquium ellipticum	19	1.094	1.111	3.874	6.079
Turpinia cochinchinensis	21	1.209	2.5	2.132	5.841
Actinodaphne bourdillonii	18	1.036	3.333	0.361	4.73
Actinodaphne malabarica	20	1.151	2.5	0.895	4.547
Syzygium cumini	34	1.957	1.111	1.215	4.284
Mallotus tetracoccus	22	1.267	1.944	0.926	4.137
Melicope lunu-ankenda	13	0.748	1.944	1.102	3.795
Erythrina stricta	48	2.763	0.556	0.171	3.49
Calophyllum polyanthum	4	0.23	0.556	2.636	3.422
Holigarna nigra	6	0.345	0.833	2.008	3.187
Ficus nervosa	4	0.23	1.111	1.763	3.104
Aphanamixis polystachya	19	1.094	1.667	0.314	3.074

Mesua ferrea	6	0.345	1.111	1.458	2.914
Cinnamomum verum	8	0.461	1.944	0.237	2.642
Gordonia obtusa	2	0.115	0.556	1.904	2.575
Antidesma montanum	6	0.345	1.111	0.779	2.235
Macaranga peltata	8	0.461	1.111	0.608	2.179
Celtis tetrandra	6	0.345	1.111	0.7	2.157
Mangifera indica	8	0.461	1.389	0.296	2.146
Olea dioica	9	0.518	1.389	0.173	2.08
Grevillea robusta	17	0.979	0.833	0.215	2.027
Trema orientalis	7	0.403	1.389	0.203	1.995
Syzygium gardneri	2	0.115	0.556	1.037	1.707
Dalbergia latifolia	5	0.288	0.556	0.844	1.687
Acrocarpus fraxinifolius	2	0.115	0.556	0.901	1.571
Ehretia canarensis	6	0.345	0.833	0.293	1.472
Bhesa indica	5	0.288	0.556	0.613	1.456
Sterculia guttata	8	0.461	0.833	0.054	1.348
Litsea sp.	3	0.173	0.556	0.613	1.341
Mallotus philippensis	4	0.23	0.833	0.18	1.243
Elaeocarpus variabilis	3	0.173	0.556	0.461	1.189
Mimusops elengi	1	0.058	0.278	0.717	1.052
Ligustrum perrottetii	5	0.288	0.556	0.208	1.051
Trichilia connaroides	3	0.173	0.833	0.022	1.028
Citrus reticulata	5	0.288	0.556	0.068	0.912
Cinnamomum malabatrum	5	0.288	0.556	0.044	0.888
Aporosa cardiosperma	5	0.288	0.556	0.044	0.887
Glochidion ellipticum	4	0.23	0.556	0.073	0.859
Cryptocarya wightiana	2	0.115	0.556	0.174	0.844
Nothopegia sp.	4	0.23	0.556	0.051	0.837
Litsea wightiana	3	0.173	0.556	0.101	0.829
Ficus hispida	2	0.115	0.556	0.098	0.768
Chukrasia tabularis	3	0.173	0.556	0.02	0.748
Elaeocarpus serratus	2	0.115	0.556	0.058	0.729
Casearia rubescens	2	0.115	0.556	0.048	0.718
Agrostistachys borneensis	2	0.115	0.556	0.032	0.703
Areca catechu	6	0.345	0.278	0.038	0.662
Filicium decipiens	2	0.115	0.278	0.262	0.655
Beilschmiedia bourdillonii	2	0.115	0.278	0.255	0.648
Ficus tsjahela	1	0.058	0.278	0.276	0.612
Kingiodendron pinnatum	1	0.058	0.278	0.251	0.587
Litsea oleoides	1	0.058	0.278	0.24	0.576
Holigarna arnottiana	2	0.115	0.278	0.171	0.564
Saraca asoca	4	0.23	0.278	0.038	0.546

Aglaia apiocarpa	2	0.115	0.278	0.12	0.513
Careya arborea	3	0.173	0.278	0.048	0.498
Persea americana	1	0.058	0.278	0.157	0.493
Artocarpus hirsutus	3	0.173	0.278	0.03	0.481
Croton laccifer	2	0.115	0.278	0.054	0.447
Meliosma pinnata	1	0.058	0.278	0.099	0.435
Dimorphocalyx glabellus	2	0.115	0.278	0.036	0.428
Dysoxylum malabaricum	1	0.058	0.278	0.089	0.425
Ligustrum sp.	1	0.058	0.278	0.089	0.425
Ficus callosa	1	0.058	0.278	0.085	0.421
Nothapodytes nimmoniana	2	0.115	0.278	0.024	0.417
Hydnocarpus alpina	1	0.058	0.278	0.061	0.396
Phyllanthus emblica	1	0.058	0.278	0.056	0.391
Xantolis tomentosa	1	0.058	0.278	0.05	0.385
Pterocarpus marsupium	1	0.058	0.278	0.041	0.377
Prunus ceylanica	1	0.058	0.278	0.04	0.375
Diospyros sp.	1	0.058	0.278	0.035	0.37
Theobroma cocao	1	0.058	0.278	0.032	0.367
Chrysophyllum roxburghii	1	0.058	0.278	0.029	0.365
Mastixia arborea	1	0.058	0.278	0.028	0.363
Grewia sp	1	0.058	0.278	0.023	0.359
Phoebe wightii	1	0.058	0.278	0.021	0.357
Ficus exasperata	1	0.058	0.278	0.019	0.355
Ficus tinctoria	1	0.058	0.278	0.015	0.35
Aglaia tomentosa	1	0.058	0.278	0.01	0.346
Litsea deccanensis	1	0.058	0.278	0.008	0.344
Dysoxylum ficiforme	1	0.058	0.278	0.008	0.343
Syzygium hemisphericum	1	0.058	0.278	0.007	0.343
Callicarpa tomentosa	1	0.058	0.278	0.006	0.342
Neolitsea fischeri	1	0.058	0.278	0.007	0.342
Bombax ceiba	1	0.058	0.278	0.003	0.338
Lepisanthes sp.	1	0.058	0.278	0.003	0.338
Leptonychia caudata	1	0.058	0.278	0.001	0.336
TOTAL	**1737**	**100**	**100**	**100**	**300**

No. Ind: Number of Individuals, **RD**: Relative Density, **RF**: Relative Frequency, **R. Dom**: Relative Dominance, **IVI**: Important Value Index

From the pooled data, *Vernonia arborea* was found to be the most important species with an IVI of 37.57 followed by *Artocarpus heterophyllus* (34.06) and *Toona ciliata* (23.48). *Vernonia arborea* had the highest number of individuals in the study plots (410 nos) followed by *Artocarpus heterophyllus* (344 nos) and *Toona ciliata* (160 nos).

These three species in total contributed about 52.62% of the total individual trees in CHR. The species showing most relative frequency in CHR is *Toona ciliata* (5.83) followed by *Vernonia arborea* (5.56) and *Artocarpus heterophyllus* (5.56). The most dominant species in CHR was *Persea macrantha* with a relative dominance of 8.99 followed by *Artocarpus heterophyllus* (8.70) and *Toona ciliata* (8.43). *Persea macrantha* became the most dominant species because of its high GBH. 77 species showed relative dominance below one.

Among the shade trees present in CHR, about 24.24 per cent of the species are endemic with a relative dominance of 19.01 in which 12 species of trees are endemic to south Western Ghats and 7 species are endemic to Western Ghats. Seven tree species come under IUCN red list category with a relative dominance of 2.16; they are *Actinodaphne malabarica, Dalbergia latifolia, Kingiodendron pinnatum, Saraca asoca, Aglaia apiocarpa, Dysoxylum ficiforme* and *Neolitsea fischeri. Kingiodendron pinnatum* is the only endangered species recorded from CHR.

4.2. Germination percentage of selected dominant trees

Persea macrantha, Artocarpus heterophyllus, Toona ciliata, Vernonia arborea and *Cullenia exarillata* are the trees selected as dominant trees for study since these four species had relative dominance above eight. *Cullenia exarillata* is also included in the study since it is considered as key stone species in this area. Besides this *Cullenia exarillata* is a dominant species in the undisturbed mid elevation tropical evergreen forests of south Western Ghats. *Cullenia exarillata* had the highest germination percentage (95%) followed by *Artocarpus heterophyllus* (93%), *Toona ciliata* (89%), *Vernonia arborea (89%)* and *Persea macrantha* (53%) (Table – 2.2).

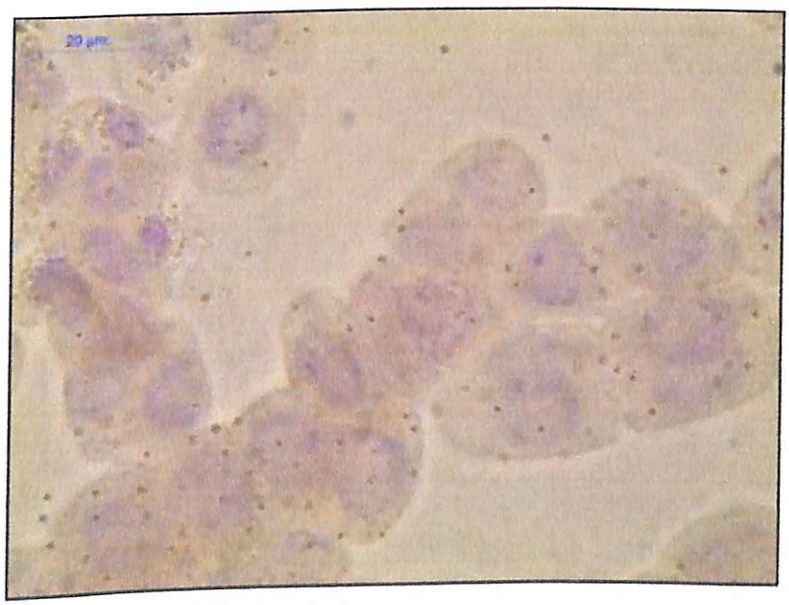
Table - 2.2 : Germination percentage of selected dominant trees

Species	No of seeds used for the study	No seeds germinated	Germination (%)
Artocarpus heterophyllus	100	93	93%
Persea macrantha	100	53	53%
Toona ciliata	100	89	89%
Cullenia exarillata	100	95	95%
Vernonia arborea	100	89	89%

4.3. Karyotype analysis

Karyotype analysis was conducted in *Persea macrantha, Toona ciliata, Cullenia exarillata, Vernonia arborea* and *Artocarpus heterophyllus.* This test was conducted to trace out whether there is any karyotype abnormality in root tip because of the excess use of pesticides in Cardamom Hill Reserves. From the study, only ball metaphase was noted in *Artocarpus heterophyllus* (Figure – 2.2). In other species the studies did not show any abnormalities. Ball metaphase is a form of mitosis with characteristically clumped chromosomes. It indicates direct destructive effects of pesticides at the chromosome level

Figure – 2.2: Ball metaphase in *Artocarpus heterophyllus*



4.4. Photosynthetic efficiency test

Photosynthetic efficiency analysis was conducted on *Persea macrantha, Toona ciliata, Cullenia exarillata, Vernonia arborea* and *Artocarpus heterophyllus*. Initially selected a saturation light level for each species and it was found that in *Persea macrantha, Toona ciliata, Vernonia arborea* and *Cullenia exarillata* the saturation light level was 2500 $\mu molm^{-2}s^{-1}$ and for *Artocarpus heterophyllus* saturation light level was 2000 $\mu molm^{-2}s^{-1}$ (Table–2.3). Photosynthetic efficiency analysis conducted on the

seedlings raised from the seeds collected from CHR did not show any stress under the controlled condition. The trial with the application of pesticide also did not show any effect of pesticide in the photosynthetic efficiency of the selected tree species (Table - 2.4). This indicates that the application of pesticide does not induce any stress on seedlings of the studied species.

Table - 2.3 : Fv/fm value of experiment on pesticide applied plants

	Artocarpus heterophyllus	**Light level ($\mu molm^{-2}s^{-2}$)**	**Fv/fm Before 1st application**	**Fv/fm After 1st application**	**Fv/fm Before 2nd application**	**Fv/fm After 2nd application**
Pesticide doses	Half the recommended dosage	1500	0.83	0.829	0.835	0.812
		2000	0.83	0.831	0.832	0.826
		2500	0.83	0.825	0.829	0.811
	Recommended dosage	1500	0.83	0.829	0.84	0.821
		2000	0.82	0.815	0.815	0.812
		2500	0.835	0.831	0.837	0.819
	Double the Recommended dosage	1500	0.8	0.805	0.798	0.833
		2000	0.83	0.825	0.828	0.829
		2500	0.83	0.83	0.832	0.825
	control	1500	0.839	0.83	0.839	0.827
		2000	0.83	0.829	0.827	0.835
		2500	0.817	0.819	0.817	0.818
	Cullenia exarillata	**Light level ($\mu molm^{-2}s^{-2}$)**	**Fv/fm Before 1st application**	**Fv/fm After 1st application**	**Fv/fm Before 2nd application**	**Fv/fm After 2nd application**
Pesticide doses	Half the recommended dosage	1500	0.798	0.795	0.816	0.827
		2000	0.805	0.81	0.819	0.831
		2500	0.801	0.805	0.811	0.819
	Recommended dosage	1500	0.819	0.82	0.819	0.823
		2000	0.82	0.815	0.823	0.824
		2500	0.809	0.82	0.803	0.837
	Double the Recommended dosage	1500	0.816	0.8	0.817	0.807
		2000	0.83	0.815	0.836	0.824
		2500	0.82	0.825	0.808	0.826
	Control	1500	0.79	0.8	0.795	0.819
		2000	0.8	0.775	0.801	0.798
		2500	0.801	0.793	0.818	0.825

Table -2.4 : Photosynthetic efficiency analysis in selected tree species

Saturation light level for *Artocarpus heterophyllus*		
Light level (µmolm^{-2}s^{-2})	**Mean (fv/fm)**	**Mean (fv/fm) control**
1500	0.759	0.754
2000	0.767	0.761
2500	0.754	0.767
Saturation light level for *Persea macrantha*		
Light level (µmolm^{-2}s^{-2})	**Mean (fv/fm)**	**Mean (fv/fm) control**
1500	0.751	0.776
2000	0.742	0.746
2500	0.751	0.749
Saturation light level for *Toona ciliate*		
Light level (µmolm^{-2}s^{-2})	**Mean (fv/fm)**	**Mean (fv/fm) control**
1500	0.680	0.700
2000	0.742	0.745
2500	0.719	0.716
Saturation light level for *Vernonia arborea*		
Light level (µmolm^{-2}s^{-2})	**Mean (fv/fm)**	**Mean (fv/fm) control**
1500	0.751	0.749
2000	0.713	0.715
2500	0.743	0.740
Saturation light level for *Cullenia exarillata*		
Light level (µmolm^{-2}s^{-2})	**Mean (fv/fm)**	**Mean (fv/fm) control**
1500	0.613	0.610
2000	0.644	0.641
2500	0.786	0.789

5. Discussion

5.1. Dominant trees

A total of ninety nine trees species were recorded from the study area of about 5.5 ha of sample plots which was approximately about 11.24% of tree species in Kerala. In a study, 740 species of flowering plants were collected and identified from Mathikettan shola national Park which comes under the boundary of Cardamom

Hill Reserves (Jomy, 2011)). A total of 1440 species of flowering plants were identified from Periyar Tiger Reserve (Sasidharan, 1998). The understory forest of Cardamom track of CHR has been replaced by cardamom monoculture. From the study plots of CHR, 99 species of trees were recorded. A number of invasive species were also recorded from this area which include *Grevillea robusta, Erythrina subumbrans,* etc. Trees are the only main representatives of past evergreen forest community that existed in this area. A majority of the forest tree species have been replaced by favorable and fast growing tree species like *Artocarpus heterophyllus, Vernonia arborea, Toona ciliata,* etc. As per previous reports *Cullenia exarillata, Mesua ferrea* and *Palaquim ellipticum* were the dominant tree species in mid elevation evergreen forests of South Western Ghats (Pascal, 2004). From the present study in CHR, *Persea macrantha* has been found to be the dominant tree species with a relative dominance of 8.991 followed by *A. heterophyllus* (8.699), *T. ciliata* (8.431) and *V. arborea* (8.405).

Western Ghats show high degree of endemism. Fifty six percentage of evergreen trees in Western Ghats are endemic (Myers, 1988). Among the trees present in CHR about 24.24 per cent of species are endemic to Western Ghats. The results are in confirmation with earlier studies which show that 1272 species of plants are considered endemic to South Western Ghats. Studies on the flora of Periyar Tiger Reserve placed 515 endemic species and 150 species under various threat categories (Sasidharan, 1998). From the study area (CHR) only seven tree species are recorded in various threat categories in which six are vulnerable and only one endangered (*Kingiodendron pinnatum*). At Mathikettan Shola National Park, 346 species are endemic to South Western Ghats and 48 species come under various threat categories (Jomy, 2011). Mathikettan Shola National Park is an undisturbed forest community coming under CHR. This shows the degraded status of evergreen forest in this area.

Intensive cultivation of cardamom leads to a high degree of simplification and homogenisation of the highly diverse evergreen forest of CHR. Introduction of new varieties of cardamom and change in land use pattern highly affect the forest tree canopy of this area. Lack of natural regeneration and introduction of economic and farmer friendly tree species highly affect the diversity of forest shade tree species. Fast growing tree species like *Artocarpus heterophyllus, Vernonia arborea, Toona ciliate,* etc. are mainly planted as shade trees by the farmers in the plantations. Dominant tree species of evergreen forests of South Western Ghats like *Cullenia exarillata, Palaquium ellipticum,* etc. are disappearing from this area.

5.2. Karyotype analysis

Pesticides may cause metabolic imbalance, which may interfere with the synthesis state and structure of nucleic acid, which in turn may cause physiological effects and structural changes in the chromosomes during cell division that may lead to mitotic delay and mitotic inhibition. In the present study, one of the most frequent chromosome abnormalities noticed was the characteristically clumped chromosomes at metaphase stage of mitosis called ball metaphase observed in *Artocarpus heterophyllus.* Ball metaphase results from the complete destruction of spindle fibers and a subsequent clumping of chromosomes into a tight ball. This indicates the direct destructive effects of pesticides on the chromosome level (Renjana et al., 2013).

5.3. Photosynthetic efficiency test

Photosynthetic efficiency test is mainly done to identify whether the plant is under stress or not. From the present study, it is noticed that there is no direct effect of pesticides on photosynthetic efficiency in the plants studied. Since the current study is based on experimental studies under controlled conditions and further studies are desirable to understand the effect of pesticides under stress induced by other environmental factors also.

Section -3

Soil microbes of Cardamom Hill Reserves and their role in pesticide degradation

1. Introduction

Pesticides are used to control the damages of pest for commercially important crops in agriculture field. Among the pesticides use of chlorpyrifos is at the top of the list of organophosphorus compounds in the Indian market. Chlorpyrifos is an organophosphorus insecticide and reported as toxic chemical as it show mutagenic and carcinogenic effects on organisms. Most of the organisms die due to toxicity of pesticides but few of them evolve in different ways and use pesticide compounds in metabolism (Horvath, 1972; Hussain et al., 2007 and Lakshmi et al., 2009).

Pesticides may affect soil microbial populations, stimulating growth of certain microorganisms and exerting toxic effects and inhibiting growth of others. So, identification and characterization of these microbial species is important to explore the potential of bioremediation. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. Therefore, in present investigation an attempt has been made to isolate and characterize microbes from pesticide polluted sites.

Successful removal of pesticides by the addition of bacteria (bioaugmentation) had been reported earlier for many compounds, including chlorpyrifos, endosulfan, parathion, coumaphos, ethoprop and atrazine (Singh et al., 2004). Only specific microorganisms which are tolerant to pesticides may remain and further multiply. Use of bacterial strain to degrade the pesticides - chlorpyrifos and quinalphos will be helpful to make toxic free agriculture practices. Insecticides and their degradation products generally get accumulated in the top soil and influence not only the population of various groups of soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation. Microorganisms play an important role in degrading synthetic chemicals in soil. They have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. The metabolism of chlorpyrifos by microorganisms in soil has been reported with 3, 5, 6-trichloro-2-pyridinol (TCP) as the primary breakdown product. Use of pesticide degrading microbial systems for bioremediation, thus, receives attention because of its cost effectiveness and ecofriendly nature.

2. Materials and Methods

2.1 Soil sample collection

Soil samples were collected in Randomized Complete Block Design (RCBD). Four soil samples were collected from each grid at a depth of 0-10 cm and mixed together to get one composite sample. The samples were air dried and stored at 4°C for the further studies.

2.2 Isolation and characterization of microbes from pesticide contaminated soil

Soil dilution plate method have used and appropriate dilutions - 10^{-3} for fungi, 10^{-4} for actinomycetes and 10^{-5} for bacteria were prepared. Potato Dextrose Agar (PDA) and Rose Bengal Agar (RBA) were used for isolation of fungi, while Starch Casein Agar (SCA) and Nutrient Agar (NA) were used to isolate actinomycetes and bacteria, respectively. Soil samples were also subjected to pesticide amended (100µg l^{-1}) soil dilution plating on NA and PDA. Ten grams of soil sample was taken in a conical flask containing 90ml sterile water was stirred with a magnetic shaker for 20-30 minutes. While the soil suspension was in motion, 10ml was withdrawn and added to 90ml of sterile water contained in another flask. The process was repeated until the desired dilution was obtained. One millilitre aliquot of the desired dilution was aseptically pipetted out into sterilized petridishes containing 12-15 ml of molten NA or PDA. Petridishes were gently swirled in clockwise and anti-clock wise direction to disperse the diluted soil suspension on the medium. The spread plates were incubated at 12/12 h regimes of light and darkness at 23±2°C for five to seven days and extended to 25 days for fungi (for pesticide amended), for bacteria plates were incubated at 35±2°C for 48 h and extended to 96h (for pesticide amended) and actinomycetes plates were incubated at 35°C for 10 to 14 days.

2.3 Identification of bacterial and fungal isolates

Individual bacterial colony cultured on NA medium was taken from the growing margin with a fine tipped sterile loop and incubated for 36-48h to obtain the pure culture. The identification of bacterial isolates were carried out by the conventional bacteriological methods by studying colony morphology, staining methods, endospore staining, motility and by performing biochemical tests using Bergey's manuals (Buchnan and Gibbons, 1975).

For identification of fungi, individual hyphal tips of the fungal colony cultured on PDA were taken from the growing margin with a sterile needle and inoculated on the PDA medium and incubated for five to seven days to obtain the pure culture. The fungal species were identified based on colony morphology characteristics,

hyphae, fruiting bodies, conidia/spore colour, shape and size using identification manuals (Arx, 1981; Barnett, 1972; Booth, 1971; Ellis, 1976; Ramarao and Manoharachary, 1990; Subramanian, 1983 and Sutton, 1980).

2.4 Screening of microbes for pesticide resistance *in vitro*

Standardization of Pesticide Concentration

Various concentrations of pesticides such as chlorpyrifos & quinalphos were taken and amended individually to plates with media at 50, 100, 200, 300, 400 µg/ml. It was helpful to check out the efficacy of microbes to degrade the pesticide and also maintained the control plates for each concentrations without pesticide. Serially diluted soil samples were taken and inoculated on each plate and the plates (NA) were incubated at 30°C for 3 days for bacteria and the fungal plates (PDA) were incubated at 27±2°C for seven days and the colonies were observed for morphological identification. Developed colonies were isolated and purified further by transferring them into the respective medium that contain the same concentration of pesticide (50, 100, 200, 300, 400µg/ml). Nutrient agar and Potato Dextrose Agar plates incubated at 30°C for 3 days and 27 ± 2°C for seven days for bacteria and fungi respectively were extended up to 30 days for observation of clear zones for pesticide degrading microorganisms. Based on the appearance of clear zones around the colonies the putative positive strain were selectively isolated and were further purified by transferring them to synthetic agar media plates supplemented with different concentration of pesticide several times at respective temperatures for 30 days or more days needed as they show the degradation property. The isolated bacterial and fungal strains were identified based on morphological and biochemical methods.

2.5 Determination of biomass/growth of bacterial and fungal isolates on minimal salt (Nutrient and Potato Dextrose) broth

The suspension of 24-48 hour old cultures of bacteria (*Bacillus licheniformis, Bacillus subtilis* and *Micrococcus varians*) and 4-5 days old cultures of fungal (*Aspergillus niger, Blastomyces dermites, Cladosporium herbarum, Colletotricum gloesporioides, Paecilomyces* sp., and *Penicillium crysogenum*) isolates were used to prepare the inoculum. A loop full of above bacterial and fungal cultures were inoculated into 50 ml of nutrient broth and PDB respectively, amended with different concentrations (50 - 400 µg/ml) of two different pesticides - chlorpyrifos and quinalphos and flasks were incubated at 37°C for bacteria for 3 days and 27±2 °C for fungi for 7 days in an orbital shaker at 105 rpm. Bacterial culture flasks were centrifuged at 5000rpm for 10 minutes. The supernatant was discarded and the pellet was collected and weighed to evaluate the

bacterial biomass/growth. In case of fungi cultures were filtered through triple layered muslin cloth and mycelia mats (cultures) were dried in hot air oven at 60°C for 48 hours and weighed to evaluate the fungal biomass/growth. Control has maintained for both bacteria and fungi with respective medium without pesticides.

2.6 Effect of carbon and nitrogen sources for the biomass/growth of pesticide degrading bacterial and fungal isolates

The effect of additional supply of carbon and nitrogen sources were tested for the maximization/enhancement of growth of pesticide biodegrading microbial isolates. The bacterial and fungal isolates were cultivated in 200 ml of NA and PDB respectively, amended with 100 µg/ml of pesticides and additional supply of various carbon and nitrogen sources (Peptone-0.5, Beef extract- 1.0, $NaNo_3$ - 1.0, Glucose - 5.0 and Sucrose - 10.00 g/l) and incubated at respective temperatures for 6 days (bacteria) and 12 days (fungi). In case of bacteria - *Micrococus varians*- initial samples were drawn at 0 hours and remaining samplings were drawn at an interval of 2 days (48 hours) up to eight days and evaluated the growth of bacterial biomass using UV - spectrophotometer at 600nm. In case of fungi, biomass was estimated by dry weight of fungal mycelia.

3. Results and Discussion

Excessive use of pesticides for the agriculture purposes over the years have led to many problems of biological systems in the environment. By considering the toxicity of the compound it is essential to remove them from the environment. Biological removal of the pesticide is the easiest way as the microorganisms can use such hazardous compound and convert them into nontoxic metabolites. Seventeen grid samples were subjected to microbial analysis. There were about 169 isolates belonging to 25 genera consisting 55 species of fungi, 40 bacterial isolates and 25 different types of actinomycetes (Table 3.1-3.3).

Table 3.1. Fungi isolated from the CHR soil samples

Sl. No.	Fungi isolated	Sl. No.	Fungi isolated
1	*Alternaria alternata*	2	*Alternaria tenuous*
3	*Alternaria sp 1 - 2*	4	*Aspergillus flavus*
5	*Aspergillus niger*	6	*Aspergillus candida*
7	*Aspergillus ochraceus*	8	*Aspergillus* sp. 1- 5
8	*Cladosporium cladosporoidies*	9	*Cladosporium herbarum*
10	*Cladosporium robusta*	11	*Chaetomium globosum*
12	*Chaetomium* sp. 1 - 2	13	*Fusarium oxysporum*
14	*Fusarium* sp. 1- 7	15	*Rhizopus stolonifera*
16	*Rhizopus* sp. 1- 3	17	*Mucor* sp.
18	*Cephalosporium* sp.	19	*Penicillium chrysogenum*
20	*Penicillium citrinum*	21	*Penicillium* sp 1 – 21
22	*Trichoderma harzianum*	23	*Trichoderma viride*
24	*Trichoderma* sp. 1- 4	25	Non-sporulating fungal isolates 1 - 8
26	Unidentified fungal isolates 1-15		

Table 3.2. Bacterial isolates from the CHR soil samples

Sl. No.	Bacteria isolated	Gram Staining	Shape
1	Bacterial Isolate 1-5, 13, 16, 20, 21, 24, 27-31, 35, 38, 39	+	Rod
2	Bacterial Isolate 6-12, 15, 19, 22, 25, 32-34, 36	+	Round
3.	Bacterial Isolate 14, 17, 18, 23, 26, 37, 40	+	Spiral

Table 3.3. Actinomycetes isolated from the CHR soil samples

Sl. No.	Actinomycetes isolated	Colour and morphology
1.	Unidentified isolate 1 - 2	White small round
2.	Unidentified isolate 3 - 4	Greenish brown round
3.	Unidentified isolate 5 - 6	Yellow small round
4.	Unidentified isolate 7-8	Brown medium round
5.	Unidentified isolate 9-10	Red colonies
6.	Unidentified isolate 11-12	White grayish wrinkled
7.	Unidentified isolate 13-14	Yellow powdery
8.	Unidentified isolate 15-16	Grayish to black wrinkled
9.	Unidentified isolate 17-18	Pale yellow small colony
10.	Unidentified isolate 19-20	Black small colonies
11.	Unidentified isolate 21-22	Pale powdery spherical
12.	Unidentified isolate 23-24	Yellow small colony
13.	Unidentified isolate 25	Reddish brown colony

3.1 Identification of pesticide resistant and degrading bacterial and fungal isolates

Out of a total of 40 bacterial isolates, four showed resistance and degradation property in different pesticide concentrations which was confirmed by the maximum clear zone formation around the colonies, out of four colonies only three were identified up to the species level and due to the time constraints we could not able to do the one unidentified bacteria based on colony morphology, Gram staining and biochemical tests (Table -3.4 and Figure - 3.1.) using Bergey's Manual of Determinative Bacteriology (Buchnan and Gibbons, 1975) of the three isolates were identified as *Bacillus licheniformis, Bacillus subtilis* and *Micrococcus varians* (Table-3.4) and identification of fungi was carried out by Lacto Phenol Cotton Blue Method.

Table 3.4. Biochemical tests conducted for bacterial isolates with pesticide degradation capacity

Sl. No.	Isolate No.	Procedure	Inference	Result
1	T 1	Gram staining: to identify positive and negative bacteria.	Gram positive bacteria appear violet/purple. Gram negative bacteria appear pink.	Gram positive rod
		Endospore staining: to understand the spore stage of bacteria	Vegetative cell appear pink/red Endospore should be seen as green ellipses.	Spores: green colour
		Starch hydrolysis test: This test is used to identify bacteria that can hydrolyze starch (amylose and amylopectin) using the enzymes alpha-amylase and oligo-1,6-glucosidase. Often used to differentiate species from the genera *Clostridium* and *Bacillus*.In order to interpret the results of the starch hydrolysis test, iodine must be added to the agar. The iodine reacts with the starch to form a dark brown color. Thus, hydrolysis of the starch will create a clear zone around the bacterial growth.	Transparent clear zone formed around colonies of bacteria indicates starch hydrolysis positive. Zones not formed around colonies of bacteria indicates starch hydrolysis test negative.	Positive
		Voges-Proskauer:This test is used to determine which fermentation pathway is used to utilize glucose. Methyl Red / Voges-Proskauer (MR/VP) broth prepared and culture was inoculated and incubated for 24-48 hrs. Barrits reagent A (12 drops) and Barrits reagent B (6 drops) was added and colouration was noted after half an hour. Red colour shows positive result otherwise no colouration.	Development of red colour indicate VP positive. Yellow colour indicates VP negative.	Red colouration: positive result.
		Simmon's Citrate Agar: This is a defined medium used to determine if an organism can use citrate as its	Green citrate medium turns to blue indicates	Blue colour: positive result

		sole carbon source. The alkaline pH turns the pH indicator (bromthymol blue) from green to blue. This is a positive result. Otherwise no colouration.	positive test. No change in colour (green) indicates negative.	
		Nitrate Broth: This is a differential medium. It is used to determine if an organism is capable of reducing nitrate (NO_3^-) to nitrite (NO_2^-) or other nitrogenous compounds via the action of the enzyme nitratase (also called nitrate reductase). These tubes are first inspected for the presence of gas in the Durham tube. To this, culture was added and incubated for 48 hrs. After that sulfanilic acid (often called nitrate I) and dimethyl-alpha-napthalamine (nitrate II) was added. Red colour shows positive result otherwise no colouration.	Red colour considered as positive result. No colouration indicates negative result.	Red colour: positive result
		6.5% NaCl: to the nutrient agar medium 6.5% NaCl in 100ml (peptone, NaCl: 0.5g, beef extract: 0.3g, agar: 2g,).	A positive salt tolerance test is indicated by growth and/or turbidity in media. No growth / absence of turbidity indicated negative result.	Positive
		Hichrome Bacillus Agar	Yellowish green to green colonies	Positive
		The unknown (T1) degrading bacteria is *Bacillus subtilis*		*Bacillus subtilis*
2	T2	Gram staining: to identify positive and negative bacteria.	Gram positive bacteria appear violet/purple. Gram negative bacteria appear pink.	Gram positive coccus
		Catalase test: This test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide by	The bubbling resulting from production of oxygen gas	Positive result

		breaking it down into water and oxygen gas. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result.otherwise no bubbles occurred.	indicates as positive. No bubbling indicates negative.	
		Manittol fermentation test: An inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red mannitol broth The inoculated tube is incubated at 35-37^0 C for 24 hours and the results are determined. A positive test consists of a color change from red to yellow, indicating a p*H* change to acidic.	A colour change from red to yellow considered positive. No colour change (red) as negative.	Negative result no colouration occurred.
		Mannitol salt agar (MSA): This type of medium is both selective and differential. The MSA will select for organisms such as *Staphylococcus* species which can live in areas of high salt concentration. Some species of *Micrococcus*, such as *M. luteus* (yellow) and *M. roseus* (red) produce yellow or pink colonies when grown on mannitol salt agar.	Medium turns yellow indicates positive. Medium does not change colour indicates negative.	Positive yellow pigment colony
		Glucose fermentation test: An inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red glucose broth. The inoculated tube is incubated at 35-37 C for 24 hours and the results are determined. A positive test consists of a colour change from red to yellow, indicating a pH change to acidic.	A colour change from red to yellow indicates positive. No colour change indicate negative.	Positive yellow colour, acid production.
		The unknown degrading (T2) sample is *Micrococcus varians*		*Micrococcus varians*
3	T3	Gram staining: to identify positive and negative bacteria.	Gram positive bacteria appear violet/purple. Gram negative bacteria appear pink.	Gram positive rod
		Endospore staining: to understand	Vegetative cell	Spores: green

		the spore stage of bacteria.	appear pink/red Edospore should be seen as green ellipses.	colour
		Starch hydrolysis test: This test is used to identify bacteria that can hydrolyze starch (amylose and amylopectin) using the enzymes a-amylase and oligo-1,6-glucosidase. Often used to differentiate species from the genera *Clostridium* and *Bacillus*.In order to interpret the results of the starch hydrolysis test, iodine must be added to the agar. The iodine reacts with the starch to form a dark brown color. Thus, hydrolysis of the starch will create a clear zone around the bacterial growth.	Transparent clear zone formed around colonies of bacteria indicates starch hydrolysis positive. Zones not formed around colonies of bacteria indicates starch hydrolysis test negative.	Positive
		Voges-Proskauer:This test is used to determine which fermentation pathway is used to utilize glucose. Methyl Red / Voges-Proskauer (MR/VP) broth prepared and culture was inoculated and incubated for 24-48 hrs. Barrits reagent A (12 drops) and Barrits reagent B (6 drops) was added and colouration was noted after half an hour. Red colour shows positive result otherwise no colouration.	Development of red colour indicate VP positive. Yellow colour indicates VP negative.	Red colour positive
		Simmon's Citrate Agar: This is a defined medium used to determine if an organism can use citrate as its sole carbon source. The alkaline pH turns the pH indicator (bromthymol blue) from green to blue. This is a positive result. Otherwise no colouration.	Green citrate medium turns to blue indicates positive test. No change in colour (green) indicates negative.	Blue colour Positive
		Nitrate Broth: This is a differential medium. It is used to determine if an organism is capable of reducing nitrate (NO_3^-) to nitrite (NO_2^-) or other nitrogenous compounds via the action of the enzyme nitratase (also called nitrate reductase). These	Red colour considered as positive result. No colouration indicates negative result.	Red colour Positive

		tubes are first inspected for the presence of gas in the Durham tube. To this, culture was added and incubated for 48 hrs. After that sulfanilic acid (often called nitrate I) and dimethyl-alpha-napthalamine (nitrate II) was added. Red colour shows positive result otherwise no colouration.		
		6.5% NaCl: to the nutrient agar medium 6.5% NaCl in 100ml (peptone, NaCl: 0.5g, beef extract: 0.3g, agar: 2g,). Growth at 55°C	A positive salt tolerance test is indicated by growth and/or turbidity in media. No growth / absence of turbidity indicated negative result. No growth / growth at 55°C	Positive Growth/turbidity observed Positive
		Hichrome Bacillus Agar:	Yellowish green to green colonies.	Positive
		The unknown (T3) degrading bacteria is *Bacillus subtilis* or *Bacillus licheniformis.*		*Bacillus licheniformis*

Yellow pigmentation on MSA

Oxidase Test

Gram Staining

Figure - 3.1. Identification of bacterial isolates by Gram staining and biochemical tests

Out of 169 fungal isolates, 61 showed resistance and degradation property by clear zone formation and other colonies showed pesticide resistance. Out of 61 species among pesticide resistant and degrading candidate isolates - *Aspergillus niger, Blastomyces dermites, Cladosporium herbarum, Collectotricum gloesporioides, Paecilomyces* sp., and *Penicillium crysogenum* showed more prominent resistance by colony growth and had developed maximum clear zone formation are selected for further studies.

Two soil samples were also subjected to pesticide - Chlorpyrifos and quinalphos amended (100µg l^{-1}) soil dilution plate on NA and PDA for tracing the pesticide resistance and degradation effect of bacteria and fungi. About five isolates, belonging to two genera and three species of fungi (*Aspergillus flavus, A. niger* and *Penicillium* sp.,) and two bacterial isolates were found to have chlorpyrifos degrading capacity. Among five fungal isolates, *Aspergillus niger* and two bacterial isolates (isolate 01 and 02) showed tendency of pesticide resistance and have shown pesticide degradation by developing clear zone around the microbial isolates. In case of quinalphos amended medium about 11 isolates belong to genera of fungi and three bacterial isolates were found. Among the isolates only one fungal species, *Aspergillus niger* showed efficacy of pesticide resistance and showing pesticide degradation by developing a clear zone and no bacterial species showed their resistance against pesticides and degradation properties.

3.2 Screening of bacterial and fungal isolates for pesticide resistance and degradation *in vitro*

Among the isolates, 169 fungal and 40 bacterial colonies were found to be pesticide resistant. All the isolates were screened for their ability to resist chlorpyrifos and quinalphos at different concentrations ranging from 50 to 400 µg/ml. Out of 40 bacterial isolates; four isolates showed resistance and degradation property by developing a clear zone around the colonies and other isolates showed pesticide resistance only. Among the four bacterial isolates prominent three were tested for biomass estimation and increased biomass was observed up to 100µg/ml concentration against chlorpyrifos and quinalphos and biomass decreased in higher pesticide concentration. In case of fungal isolates out of 169 fungal isolates, 61 were found to have resistance and degradation property by showing a clear zone around the colonies but the other colonies showed only pesticide resistance. Clark and Wright (1970) reported and isolated *Arthrobacter* and *Achromobacter*, which utilized a herbicide (isopropyl N -phenylcarbamate) from soil and suggest that the process of clear zone formation was the gradual dissolution and diffusion of the

herbicide/pesticide in the culture medium. These processes of formation of clear zone by these strains were similar to our observation. The resistant bacterial isolates were identified on the basis of morpho-cultural and biochemical considerations (Table 3.2 and 3.4). Out of four, three isolates viz., *Bacillus licheniformis, Bacillus subtilis, Micrococcus varians,* and one unidentified sp. offered resistance up to 100µg/ml. Yun Long et al., (1997) also reported the isolation and identification of bacteria from soils, capable of degrading a number of pesticides.

3.3 Biomass/growth evaluation of pesticide degrading bacterial and fungal isolates on minimal salt (Nutrient and Potato Dextrose) broth amended with different concentrations of pesticides

Pesticide degrading bacteria and fungi were isolated and identified from pesticide contaminated soils of CHR. The isolated and identified bacteria (*Bacillus licheniformis, Bacillus subtilis* and *Micrococcus varians)* and fungi (*Aspergillus niger, Blastomyces dermites, Cladosporium herbarum, Colletotricum gloesporioides, Paecilomyces* sp., and *Penicillium crysogenum)* were assessed in nutrient broth and potato dextrose broth respectively, amended with different concentrations of pesticides (50 – 400 µg/ml) - chlorpyrifos and quinalphos and incubated at 37°C for bacteria for three days and 27 ± 2 °C for fungi for 7 days. Among the three bacterial isolates, *M. varians* showed effective resistance and maximum biomass/growth followed by *B. licheniformis* and *B. subtilis* (Table - 3.5). Among the fungi, *A. niger* showed the maximum biomass/growth and also reduced the turbidity of broth followed by *P. crysogenum, C. herbarum, Paecilomyces* sp., *C. gloesporioides* and *B. dermites* (Table - 3.6).

Table 3.6. Biomass/growth evaluation of fungal isolates on minimal salt broth (PDB) amended with different concentrations of Chlorpyrifos and Quinalphos at seven days of incubation

Pesticide Concentration (µg/ml)	Fungal Biomass (g/l)					
	AN	PC	CH	P sp.	CG	BD
Chlorpyrifos						
Control (C+M)	0.488	0.438	0.392	0.397	0.355	0.335
50	0.415	0.384	0.324	0.315	0.309	0.302
100	0.432	0.407	0.338	0.321	0.319	0.315
200	0.242	0.295	0.282	0.279	0.199	0.173
300	0.125	0.115	0.108	0.102	0.106	0.088
400	0.039	0.032	0.030	0.025	0.010	0.000
Quinalphos						
50	0.424	0.395	0.324	0.335	0.315	0.302
100	0.478	0.432	0.385	0.368	0.340	0.328
200	0.332	0.295	0.222	0.209	0.189	0.163
300	0.125	0.115	0.108	0.102	0.106	0.088
400	0.039	0.032	0.030	0.025	0.010	0.000

AN - *Aspergillus niger*, PC - *Penicillium crysogenum*, CH - *Cladosporium herbarum*, P sp. - *Paecilomyces* sp., CG - *Colletotricum gloesporioides* and BD - *Blastomyces dermites*.

Table 3.5. Biomass/growth evaluation of bacterial isolates on minimal salt broth (NB) amended with different concentrations of pesticides at two days of incubation

Sl. No.	Pesticide Concentration (µg/ml)	Bacterial Biomass (g/l)					
		Chlorpyrifos			Quinalphos		
		1	2	3	1	2	3
1	Control (C+M)	0.272	0.264	0.384	0.312	0.265	0.395
2	50	0.264	0.254	0.344	0.295	0.250	0.375
3	100	0.278	0.262	0.380	0.305	0.261	0.390
4	200	0.222	0.212	0.222	0.213	0.199	0.299
5	300	0.115	0.105	0.135	0.136	0.126	0.156
6	400	0.041	0.031	0.057	0.035	0.025	0.065

[1]*Bacillus licheniforms*, [2]*Bacillus subtilis* and [3]*Micrococcus varians*

3.4. Effect of carbon and nitrogen sources on the biomass/growth of pesticide degrading bacterial isolates in nutrient broth amended with and without pesticides

The growth of three pesticide degrading bacteria *B. lichiniformis, B. subtilis* and *M. varians* were assessed in nutrient broth amended with 100µg/ml of two different pesticides chlorpyrifos and quinalphos with different carbon and nitrogen sources (Peptone-0.5, Beef extract – 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l). The growth of bacteria was found to be maximum in the presence of sucrose. Among the three bacterial isolates *M. varians* showed the maximum tolerance and biomass in sucrose amended medium (Table - 3.7, 3.8 and 3.9). In case of *M. varians*, growth in second day of incubation was high in beef extract supplemented medium and after second day incubation again maximum growth was observed in sucrose supplemented medium and continued till the eighth day of incubation (Tables - 3.10 – 3.14).

Table 3.7. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Bacillus licheniformis* amended with and without pesticides (100µg/ml) -Quinalphos and chlorpyrifos OD at six days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	1.039	0.213	1.394	0.112	1.377
2	Peptone	1.001	0.208	1.420	0.102	1.367
3	Beef extract	1.068	0.304	1.347	0.202	1.381
4	Glucose	1.118	0.205	1.434	0.101	1.255
5	Sucrose	1.079	0.204	2.456	0.203	2.280
6	$NaNO_3$	1.055	0.104	1.258	0.201	1.320

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

Table 3.8. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Bacillus subtilis* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at six days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.325	0.135	0.968	0.104	0.891
2	Peptone	0.388	0.216	2.166	0.107	2.250
3	Beef extract	0.356	0.115	2.077	0.106	2.310
4	Glucose	0.289	0.189	0.507	0.109	0.445
5	Sucrose	0.430	0.157	2.267	0.108	2.881
6	$NaNO_3$	0.263	0.132	1.147	0.107	1.140

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

Table 3.9. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Micrococus varians* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at six days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.751	0.146	1.950	0.091	1.995
2	Peptone	0.450	0.214	2.080	0.072	2.038
3	Beef extract	0.385	0.178	2.345	0.098	2.382
4	Glucose	0.310	0.184	0.858	0.149	0.543
5	Sucrose	0.905	0.152	2.598	0.078	3.547
6	$NaNO_3$	0.395	0.134	1.894	0.093	1.870

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

Table 3.10. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate *Micrococus varians* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at zero days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.211	0.145	1.281	0.081	1.231
2	Peptone	0.216	0.212	1.418	0.070	1.235
3	Beef extract	0.205	0.179	1.331	0.090	1.390
4	Glucose	0.206	0.183	0.397	0.145	0.081
5	Sucrose	0.215	0.151	1.340	0.075	1.254
6	$NaNO_3$	0.201	0.135	1.235	0.097	1.245

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

Table 3.11. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Micrococus varians* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at two days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.367	0.145	2.206	0.081	1.984
2	Peptone	0.405	0.212	2.218	0.070	2.317
3	Beef extract	0.415	0.179	2.431	0.090	2.361
4	Glucose	0.356	0.183	0.797	0.145	0.281
5	Sucrose	0.381	0.151	2.210	0.075	2.254
6	$NaNO_3$	0.203	0.135	2.121	0.097	2.154

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Table 3.12. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Micrococus varians* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at four days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.364	0.145	1.364	0.081	1.971
2	Peptone	0.451	0.212	2.038	0.070	2.021
3	Beef extract	0.465	0.179	2.163	0.090	2.137
4	Glucose	0.311	0.183	0.893	0.145	0.426
5	Sucrose	0.594	0.151	2.169	0.075	2.326
6	$NaNO_3$	0.382	0.135	1.844	0.097	1.851

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Table 3.13. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Micrococus varians* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at six days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.398	0.145	1.598	0.081	1.960
2	Peptone	0.450	0.212	2.070	0.070	2.031
3	Beef extract	0.385	0.179	2.342	0.090	2.378
4	Glucose	0.310	0.183	0.853	0.145	0.539
5	Sucrose	0.905	0.151	2.594	0.075	3.537
6	$NaNO_3$	0.395	0.135	1.884	0.097	1.860

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Table 3.14. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading bacterial isolate, *Micrococus varians* amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos OD at eight days of incubation

Sl. No.	Source	Culture	Quinalphos + Medium	Quinalphos + Medium + Culture	Chlorpyrifos + Medium	Chlorpyrifos + Medium + Culture
1	NA	0.911	0.145	1.814	0.081	1.621
2	Peptone	0.412	0.212	1.879	0.070	1.848
3	Beef extract	0.377	0.179	2.077	0.090	2.054
4	Glucose	0.301	0.183	0.695	0.145	0.531
5	Sucrose	1.054	0.151	2.692	0.075	3.073
6	$NaNO_3$	0.340	0.135	1.914	0.097	1.956

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Micrococcus varians is a gram positive cocci, non motile and non spore forming usually arranged in the form of spherical cells ranging from about 0.5 to 3 micrometers in dia-meter of pairs. It shows positive for oxidase, catalase, MSA, glucose fermentation and negative to mannitol fermentation test. With above tests it was inferred that the organism is *Micrococcus varians* (Bergey's). *Bacillus subtilis* is a gram positive bacillus, facultative aerobic, non motile and spore forming bacteria. It showed positive for Starch hydrolysis, Voges-Proskauer, Simmon's Citrate Agar, Nitrate Broth, NaCl and Hichrome Bacillus Agar. From these tests it was inferred that the organism is *Bacillus subtilis. Bacillus licheniformis* is a gram positive bacillus. It is facultative aerobic and non-motile and spore forming bacteria. It gave positive for starch hydrolysis, Voges-Proskauer, Simmon's Citrate Agar, Nitrate broth, NaCl and Hichrome Bacillus Agar. From these tests it was inferred that the organism is *Bacillus licheniformis.* The isolated fungus samples were identified by lacto-phenol cotton blue method such as *Aspergillus niger, Blastomyces dermites,Cladosporium herbarum, Collectotricum gloesporioides, Paecilomyces* sp., and *P. crysogenum.*

Pesticide resistant bacterias - *M. varians, B. lichenifomis* and *B. subtilis*– were subjected to biomass/growth studies in nutrient broth amended with (100µg/ml) and without pesticides. All the three showed resistance and ability to degrade (by zone formation) quinalphos and chlorpyrifos. In the present study, the growth of three resistant bacteria *Micrococcus varians , Bacillus subtilis,* and *Bacillus licheniformis* were assessed in nutrient broth containing 100µg/ml of two different pesticides, chlorpyrifos and quinalphos with additional supply of different carbon and nitrogen sources - Peptone-0.5, Beef extract – 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNo_3$ – 1.0 g/l.

The growth of bacteria was higher in presence of sucrose, than in $NaNO_3$, beef extract and glucose. Among the three bacterial isolates, *M. varians* had the maximum biomass/growth in sucrose containing medium than others. *Micrococcus varians,B. subtilis, B. licheniformis* were selected for further studies and provided with quinalphos and chlorpyrifos in the medium at definite concentration. The lag period for each of the isolate varied, depending on the period for which the cells were grown in NB medium. The results indicate that these isolates may use quinalphos and chlorpyrifos as a source of nutrition, and also may cause enzymatic degradation or conversion to inorganic form.

Pesticides which enter the soil environment are subjected to a variety of degradation and transport processes. Overall dissipation of a pesticide from soil results from a combination of loss of mechanism such as microbial degradation, chemical hydrolysis, photolysis, volatility, leaching and surface runoff (Singh and Walker, 2006). In addition to the potential bioremedial use of microbes and enzymes for dealing with organophosphorus contamination in the environment, there has been considerable interest in the use of organophosphorus degrading enzymes prophylactically and therapeutically for organophosphorus pesticides. The extended use of chlorpyrifos for many years may permit some opportunistic microorganism to develop the capability to use this toxic compound as an energy source for their survival as reported with the organochlorine pesticides (Singh *et al.*, 2000) and with a bacterial strain capable of degrading chlorpyrifos, which was isolated from Australian soil where Chlorpyrifos had been in use for 15 years (Singh *et al.*, 2004).

It was interesting to note the ability of the bacterial strains isolated from pesticide enriched soils to tolerate and grow on higher concentration of pesticides. Many bacterial strains were isolated and grew on nutrient agar medium as well as in broth medium containing 50 ppm pesticides as the sole carbon source. Similarly, a number of researchers isolated bacteria following enrichment culture technique capable of degrading organophosphorus pesticides such as chlorpyrifos. These includes *Enterobacter* sp., *Flavobacterium* sp., *Arthrobacter* sp. and *Pseudomonas putida*. Nutrient broth is a stress free complete medium containing most of the preformed growth requirements which are directly available to the cells (Madigan et al., 1997). The generation time of all the resistant isolates in nutrient broth containing (1mg/ml and 2mg/ml) chlorpyrifos showed an extended trend because of the chlorpyrifos stress. These observations with reference to generation time under stress of chlorpyrifos in nutrient medium are in agreement with the extended generation time of soil bacteria grown in lab conditions with phenol stress (Ajaz et al., 2004).

3.6 Effect of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolates in potato dextrose broth amended with and without pesticides

The candidate fungal isolates – *Aspergillus niger, Blastomyces dermites, Cladosporium herbarum, Collectotricum gloesporioides, Paecilomyces* sp., and *P. crysogenum* were subjected to biomass/growth studies in potato dextrose broth with (100 µg/ml) and without pesticides. In case of *A. niger,* maximum growth/biomass observed in $NaNO_3$ amended medium and also notice the degradation by reduction in turbidity

(Table - 3.5). In the case of *P. crysogenum,* noticed a maximum biomass in PDB alone and PDB with other carbon and nitrogen supplements- peptone, beef extract, $NaNO_3$, sucrose and glucose showed minimum growth (Table - 3.16). *Cladosporium herbarum* gave maximum growth in PDB alone and also showed reduced turbidity and least growth was noticed in glucose containing medium (Table - 3.17). In case of *Paceiliomyces* sp. found maximum mycelia growth in broth cultures amended with $NaNO_3$ and also reduced the turbidity (degradation property). But in case of other supplements - peptone, beef extract, sucrose, glucose it was observed to yield minimum growth and noticed reduced turbidity in the sucrose containing PDB (Table 3.18). In *Colletotricum gloesporioides* maximum growth was observed in PDB amended with sucrose but in glucose negligible biomass/growth was noticed. The medium PDB amended with sucrose noticed to reduce turbidity in quinalphos (Table 3.19). In case of *Blastomyces dermites,* maximum biomass growth was observed in PDB, beef extract and sucrose and the isolates in other supplements showed low and moderate growth (Table 3.20). *Alternaria alternata, Cephalosporium* sp., *Cladosporium cladosporioides, Cladorrhinum brunnescens, Fusarium* sp., *Rhizoctonia solani,* and *Trichoderma viride,* reveal the degradation of chlorpyrifos in liquid culture (Singh and Walker, 2006). Buyanovsky et al. (1995) reported similar results of biomass in *Aspergillus* sp., *Trichederma viride* and *Penicillium* sp. grown on carbofuran and repeated application was also related to consumption of carbofuran as a sole source of carbon (Edwards et al., 1992).

Table 3.15. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolate, *Aspergillus niger* in potato dextrose broth amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos at twelve days of incubation

Sl. No.	Sources	Fungal Biomass (g/l)		
		Medium + Culture	Chlorpyrifos + Medium + Culture	Quinalphos + Medium + Culture
1	PDB	0.315	0.215	0.215
2	Peptone	0.346	0.235	0.281
3	Beef extract	0.312	0.122	0.231
4	$NaNO_3$	0.712	0.598	0.559
5	Sucrose	0.419	0.145	0.147
6	Glucose	0.445	0.139	0.132

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Table 3.16. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolate, *Penicillium crysogenum* in potato dextrose broth amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos at twelve days of incubation

Sl. No.	Sources	Fungal Biomass (g/l)		
		Medium + Culture	Chlorpyrifos + Medium + Culture	Quinalphos + Medium + Culture
1	PDB	0.638	0.492	0.428
2	Peptone	0.584	0.184	0.192
3	Beef extract	0.207	0.187	0.132
4	$NaNO_3$	0.195	0.135	0.115
5	Sucrose	0.215	0.125	0.113
6	Glucose	0.232	0.132	0.082

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

Table 3.17. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolate, *Cladosporium herbarum* in potato dextrose broth amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos at twelve days of incubation

Sl. No.	Sources	Fungal Biomass (g/l)		
		Medium + Culture	Chlorpyrifos + Medium + Culture	Quinalphos + Medium + Culture
1	PDB	0.582	0.452	0.419
2	Peptone	0.324	0.244	0.324
3	Beef extract	0.348	0.238	0.385
4	$NaNO_3$	0.382	0.282	0.322
5	Sucrose	0.308	0.123	0.138
6	Glucose	0.230	0.113	0.120

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

Table 3.18. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolate, *Paceiliomyces* sp. in potato dextrose broth amended with and without pesticides (100 µg/ml) - quinalphos and chlorpyrifos at twelve days of incubation

Sl. No.	Sources	Fungal Biomass (g/l)		
		Medium + Culture	Chlorpyrifos + Medium + Culture	Quinalphos + Medium + Culture
1	PDB	0.232	0.125	0.098
2	Peptone	0.312	0.285	0.335
3	Beef extract	0.216	0.321	0.109
4	$NaNO_3$	0.541	0.429	0.381
5	Sucrose	0.201	0.121	0.101
6	Glucose	0.142	0.021	0.015

Additional Supply to NB: Peptone-0.5, Beef extract – 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Table 3.19. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolate, *Collectotricum gloesporioides* in potato dextrose broth amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos at twelve days of incubation

Sl. No.	Sources	Fungal Biomass (g/l)		
		Medium + Culture	Chlorpyrifos + Medium + Culture	Quinalphos + Medium + Culture
1	PDB	0.455	0.255	0.213
2	Peptone	0.409	0.219	0.153
3	Beef extract	0.391	0.190	0.140
4	$NaNO_3$	0.499	0.239	0.169
5	Sucrose	0.506	0.312	0.416
6	Glucose	0.123	0.083	0.067

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose – 5.0, Sucrose – 10.0 and $NaNO_3$ – 1.0 g/l

Table 3.20. Effect of additional supply of carbon and nitrogen sources on biomass/growth of pesticide degrading fungal isolate, *Blastomyces dermites* in potato dextrose broth amended with and without pesticides (100µg/ml) - quinalphos and chlorpyrifos at twelve days of incubation

Sl. No.	Sources	Fungal Biomass (g/l)		
		Medium + Culture	Chlorpyrifos + Medium + Culture	Quinalphos + Medium + Culture
1	PDB	0.435	0.385	0.365
2	Peptone	0.302	0.211	0.302
3	Beef extract	0.382	0.315	0.328
4	$NaNO_3$	0.273	0.143	0.173
5	Sucrose	0.412	0.288	0.318
6	Glucose	0.225	0.122	0.132

Additional Supply to NB: Peptone-0.5, Beef extract- 1.0, Glucose - 5.0, Sucrose - 10.0 and $NaNO_3$ - 1.0 g/l

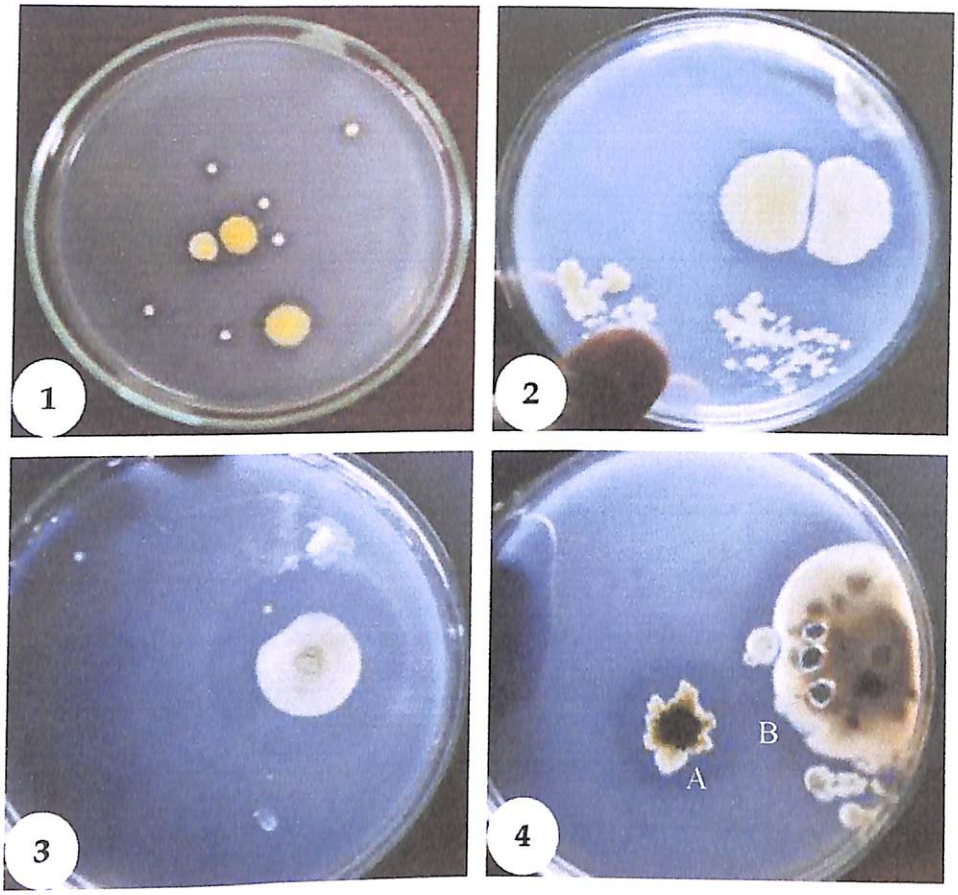


Figure - 3.2 : (1) Bacterial isolate *Bacillus licheniformis* showing clear zone formation on chlorpyrifos (2) Bacterial isolate *Micrococus varians* showing clear zone formation on quinalphos (3) Fungal isolate *Aspergillus niger* showing clear zone formation on chlorpyrifos (4) Fungal isolate *Cladosporium herbarum* (A) and *Penicillium crysogenum* (B) showing clear zone formation on quinalphos

Section -4

Role of soil chemistry in the transfer, distribution and degradation of pesticides

1. Introduction

Agriculture and environment are inextricably connected. Agricultural production and contingent food supplies world-wide are literally vital for daily human survival. Abundance of food supplies within the more industrialized nations tends to obscure the fact that the present world population can now only be sustained by the technologically-based or 'intensified' agriculture with effectively diminishing land and water resources per capita. This intensification has involved the clearance of native forests and grassland, mechanization, the introduction of selected or novel crop varieties, increasing use and dependence upon artificial irrigational and chemical aids to soil fertility, crop protection, and food harvest, storage and processing.

The goal of agricultural policy has been higher production and increased efficiency, but continued increase in production has only been possible through intensification of the farming system with a simultaneous increase of pollution risks (Winteringham, 1984). Recent trends in water pollution from agricultural nutrients and pesticides indicate that the overall pressure of agriculture on water quality in rivers, lakes, groundwater and coastal waters has eased since the early 1990s due to the decline in nutrient surpluses and pesticide use in most developed countries. Despite of this improvement, absolute levels of agricultural input pollution remain significant in many cases.

India is an agriculture-based country. Agriculture is the mainstay of our economy. That is the reason a good crop year brings buoyancy in all the sectors of the economy whereas a bad crop year results in doom. Development in agriculture is therefore of prime importance. This development in agriculture started with the green revolution in the late 1960's. High yielding varieties were introduced with increased application of chemical fertilizers and pesticides. The result was manifold increase in the yield and India has not only achieved self-sufficiency but has also become an exporter of food grains in spite of the ever increasing population. But growth in agriculture and increased food demands led to over usage of fertilizers and pesticides.

1.1. Impact of agrochemicals on environment

Agrochemicals are now a days found virtually in all natural habitats. They have severe negative effects on natural flora and fauna, biodiversity, water resources and ecosystem functioning and the equilibrium of agricultural systems. Agrochemicals can contaminate soil, water, plain lands and other vegetation. In addition to killing

insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non target plants. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms. The environmental issues due to agrochemicals can be classified based on health issues, socio economic problems, pollution of water and soil, bio diversity etc. Diffuse agricultural pollution can result in the contamination of the soil, air and water environments resulting from farming activities. Agricultural pollution is principally associated with soil particles, pesticides and other potentially toxic chemicals.

For an agricultural system to be sustainable, adverse environmental effects of agricultural production must be minimized while competitiveness and profitability are maintained or enhanced. Degradation of surface and ground water quality has been identified as the primary concern with respect to the impact of agriculture on the environment. Pesticides in groundwater are an extremely serious problem. The turnover rate for groundwater may be as a few months, but more commonly years and decades are needed to replace the water in an oxygen-free environment and such systems are much less effective in breaking down pesticide chemicals. Extremely slow dilution and breakdown means that the contaminant will be present for a long time. The most critical hazard of contaminated groundwater is the potential for toxic effects in man and domestic animals that drink the water. Contamination of an underground aquifer is also a very serious problem that cannot be easily corrected. The time taken by pesticides to travel to groundwater decreases as the depth to groundwater decreases. Generally, the depth to groundwater is least in spring and greatest in late summer. If rains come shortly after pesticide application and water table is close to the surface, a greater potential for groundwater contamination exists. The presence of pesticides in surface water, even in very small amounts, compromises the life cycle of aquatic organisms, such as algae and fish (tumors, interference with hormonal systems, respiration, growth, reproduction, etc.). Pesticides are harmful to the environment and a threat to the health of those who handle these substances, notably those working in the agriculture. But most importantly, the prolonged consumption of drinking water, fruits, and vegetables containing pesticides, even at very low doses, presents long-term risks to health.

The environmental fate of pesticides is mainly regulated by their behavior in soil where various physicochemical and biological processes control their dissipation

and movement towards other environmental compartments like air, water and biota (Figure - 4.1). The mobility of a pesticide in soil is determined by the extent and strength of sorption, which is influenced by various soil physicochemical properties. Sorption is one of the most important processes that affects the fate of pesticides in the soil and determines their distribution in the soil/water environment, it is widely used to describe the process of a pesticide partitioning between water solution and soil. Sorption also determines availability of pesticides in the soil solution that governs the amount of pesticide that is available for uptake by plants and the effectiveness of pesticides. Persistence and adsorption are the two most important characteristics of a pesticide, affecting its potential to leach to ground water. Adsorption describes how tightly a compound becomes attached to soil particles. Pesticides that are strongly adsorbed (tightly held) will be less mobile in soil that is leached with water and will be less likely to reach ground water. Some pesticides may be too tightly adsorbed to give proper pest control.

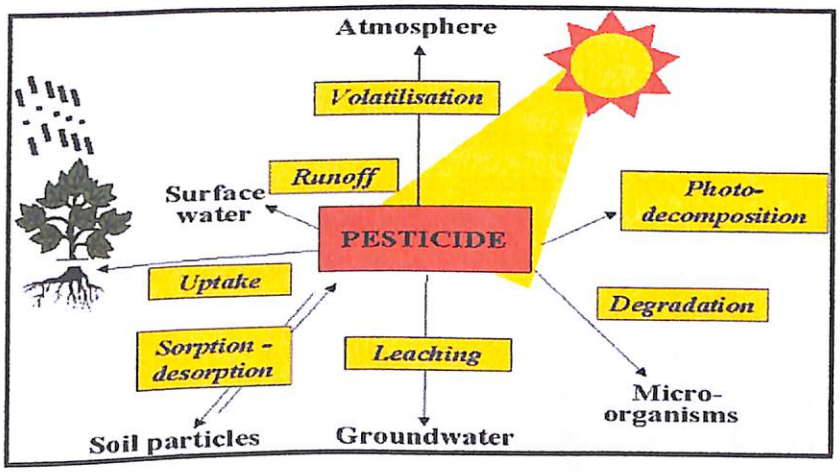


Figure - 4.1. Pesticides pathways

1.2. Pesticide consumption in India and Kerala

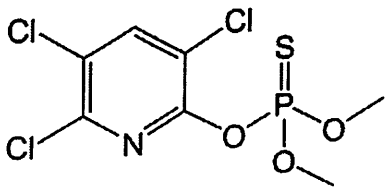
While the pesticides contribute to agricultural productivity and amelioration of vector-borne diseases, they pose serious health problems to animals and environment. There are overwhelming evidences that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment.

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. The production of pesticides started in India in 1952 with the establishment of a plant for the production of BHC near Calcutta, and India is the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to 102,240 metric tons in 1998. In 1996–97 the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which is about 2% of the total world market. The pattern of pesticide usage in India is different from that for the world in general. In India 76% of the pesticides used are insecticide, as against 44% globally. The use of herbicides and fungicides is less. The main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat (Aktar et al., 2009).
Agriculture contributes 17.2% of Kerala's economy (as of 2002-2003). Correspondingly, the sector uses a sizeable amount of pesticides (roughly 656.5 tonnes per annum), of which fungicides account for 73%. (Indira Devi, 2010). Chlorpyrifos (0,0-diethyl 0-3,5,6-trichloro-2-pyridinyl phosphorothioate), quinalphos and dimethoate are some major organophosphate pesticides used indiscriminately in Kerala (Figure - 4.2). Chlorpyrifos is stable in soils with reported half-lives ranging between 7 and 120 days. Studies have found chlorpyrifos residues in soils for over one year following application. Soil persistence may depend on the formulation, rate of application, soil type, climate and other conditions. Chlorpyrifos bound to soil may be broken down by UV light, chemical hydrolysis, dechlorination, and soil microbes. Chlorpyrifos binds strongly to soils, is relatively immobile, and has low water solubility. In contrast, its degradate TCP adsorbs weakly to soil particles and is moderately mobile and persistent in soils. Chlorpyrifos is less persistent in soils with a higher pH. Volatilization of chlorpyrifos from soil is not likely. According to a laboratory volatility study, carbon dioxide appears to be the major volatile degradate of chlorpyrifos. In this study, less than 10% of chlorpyrifos applied to soil volatilized within 30 days after application. Chlorpyrifos does not partition easily from soil to water. Therefore, chlorpyrifos found in runoff water is likely a result of soil-bound chlorpyrifos from eroding soil, rather than from dissolved chlorpyrifos. However, no drinking water standard exists for chlorpyrifos in India.

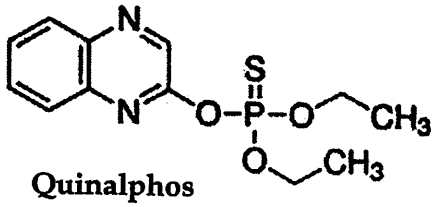
Quinalphos is another organophosphate insecticide extensively used in agriculture. Quinalphos is a hard insecticide, which has become a matter of concern because of

its potentiality and hazardous effect. Ranked 'moderately hazardous' in World Health Organization's (WHO) acute hazard ranking, use of quinalphos is either banned or restricted in most nations. Quinalphos, which is classified as a yellow label (highly toxic) pesticide in India, is widely used for the crops: wheat, rice, coffee, sugarcane, and cotton.

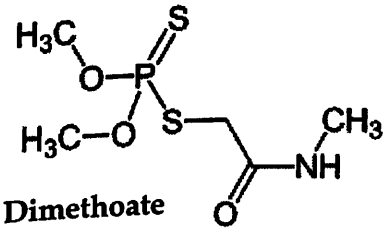
Dimethoate is an organophosphate insecticide/acaricide that is widely used on a large range of different sites. It was patented and introduced in the 1950s by M/s. American Cyanamid. Like other organophosphates, dimethoate is an anti-cholinesterase which disables cholinesterase, an enzyme essential for central nervous system function. It is used on major field crops, orchards, vegetables, ornamentals, forestry, indoor food and non-food products and treatment of sewage systems. It is a non-restricted chemical that is currently registered for use on 28 terrestrial food and feed crops, 21 terrestrial food crops, 18 terrestrial non-food crops, 9 indoor food uses, 2 indoor non-food uses, 2 outdoor residential sites, 2 forestry applications, and 1 non-food aquatic use (sewage systems).



Chlorpyrifos



Quinalphos



Dimethoate

Figure - 4.2. Chemical structure of chlorpyrifos, quinalphos and dimethoate

The leaching of pesticides to groundwater from farm lands has been of increasing concern. Differences in chemical solubility, adsorptive characteristics, volatility, and degradability as well as soil properties that effect water movement, biological activity and chemical retention all affect the amount of a pesticide that will leach to

Plate -2 : Soil sample collection from cardamom plantations in Cardamom Hill Reserves

groundwater. In order to prevent pesticide migration to groundwater, an understanding of the processes that cause pesticide migrations needs to be understood. Cardamom Hill Reserves, with its high rainfall and sloppy terrain poses a potential threat of ground water contamination with pesticides. Hence the present study was designed to understand the kinetics of major pesticides in the soils of Cardamom Hill Reserves, Kerala and thereby to reduce the potential of applied pesticides on leaching and contaminating the ground water of the region.

2. Materials and Methods

2.1. Soil collection and characterization

The collected soil samples were made free from debris, ground, air dried and sieved (2 mm sieve). The processed samples were stored for further analysis. The pH of soil was determined in 1:2.5 soil water suspensions. Organic carbon was estimated by chromic acid digestion method (Walkley and Black, 1934), and available N by alkaline permanganometry (Subbiah and Asija, 1956). Available P in the samples were extracted by Bray No. 1 followed by colorimetric estimation and available K by 1N ammonium acetate (pH 7) extraction and estimated by flame photometer. Available Ca and Mg in the samples were estimated by atomic absorption spectrophotometer after 1N ammonium acetate (pH 7) extraction (Jackson, 1958). The soil texture was estimated by Bouyoucos Hygrometer Method. Pesticide content in the samples were estimated in GC – MS with chloroform as the solvent.

2.2. Laboratory studies

For laboratory studies, surface soil samples (0 - 20 cm) were collected from Cardamom Research Station (Kerala Agricultural University), Pampadumpara. The collected samples were air-dried and ground to pass through a 2 mm sieve. These soil samples were stored in plastic bags at room temperature and used for all lab experiments.

2.2.1. Adsorption isotherms

Batch adsorption experiments were conducted to examine sorption behavior and the effects of solution pH on adsorption performance. Following a systematic process, the adsorption uptake capacity of pesticides in batch system was studied in the present work. For the experiments soil was equilibrated with three different pesticides separately. Dimethoate, chlorpyrifos and quinalphos - commonly used in the cardamom plantations - were used in this study to elucidate the soil sorption

kinetics. The pesticide solution concentrations ranged from 0 - 40 mg/ kg soil. Effects of soil reaction on adsorption was studied at two soil pH (5.4 and 3.5). All the equilibrations were done at 30^0 C for 24 hrs in a refrigerated shaker. The soil to solution ratio was maintained at 1:5 throughout the experiment. The pesticide concentrations in the supernatant solutions before and after the experiments were estimated using GC - MS (Shimadzu QP 2010).

The data obtained in batch mode studies was used to calculate the equilibrium pesticide adsorptive quantity by using the following expression:

$$qe = \frac{(Co - Ce)\, V}{m}$$

where qe is the amount of adsorbed pesticide onto per unit weight of the biomass in mg/kg, V is the volume of solution treated in litre, Co is the initial concentration of pesticide ion in mg/L, Ce is the residual pesticide concentration in mg/L and m is the mass of adsorbent in g/L (Mandal and Mayadevi, 2009).

Adsorption capacity of soils for pesticides was evaluated using the Langmuir and Freundlich adsorption isotherms. Langmuir equation is valid for monolayer sorption onto a surface with a finite number of identical sites which are homogeneously distributed over the adsorbent surface is given by the equation:

$$qe = \frac{Q_{max}.k\ C_e}{1+ k\, C_e}$$

where qe is the amount of pesticide adsorbed per unit weight of the sorbent (mg g^{-1}), C_e is the equilibrium concentration of fluoride in solution (mg L^{-1}), Q_{max} is the amount of adsorbate at complete monolayer coverage (mg g^{-1}) and gives the maximum sorption capacity of sorbent and k (Lmg^{-1}) is Langmuir isotherm constant that relates to the energy of adsorption/ binding affinity. Langmuir isotherm constants, viz., Q_{max} and k were calculated from the slope and intercept of the linear plot of C_e/q_e vs C_e.

The Freundlich sorption isotherm is given by

$$qe = K_f C_e^{1/n}$$

where K_f is the Freundlich constant indicating the sorption capacity, and n is the correction factor indicating sorption intensity (Liu and Shen, 2008)

Thermodynamic considerations of an adsorption process is necessary to conclude the spontaneity of the process. Reactions occur spontaneously at a given temperature if $\Delta G°$ (Gibbs free energy) is a negative value. The thermodynamic parameters of Gibb's free energy change, $\Delta G°$ was calculated from the following equation:

$$\Delta G° = -RT\ln k$$

where R is universal gas constant (8.314 J mol^{-1} K^{-1}) , T is the absolute temperature (K) and k is the is Langmuir isotherm constant (Lmg^{-1}).

2.2.2. Kinetic adsorption studies

For kinetic adsorption studies, 5 g of soil was mixed with 100 ml of 100 ppm standard chlorpyrifos, dimethoate and quinalphos solutions separately in a 250 ml conical flask. This mixture was shaken on an orbital shaker at a speed of 150 rpm for a period of 24 hrs at room temperature. 5 ml of supernatant solution was collected from flask at time intervals of 0, 1, 2, 4, 8 and 24 hours. The collected samples were extracted with 5 ml of chloroform in a separating funnel. The extracted samples were filtered using syringe filter and analyzed for residual pesticide concentration using GC -MS. The data was used for analyzing different kinetic models

Pseudo first order Kinetic model

First order rate constant was calculated by the following equation

$$\log (Q_{max} - Q_t) = \log Qmax - K_1.t/2.303$$

Where Q_{max} and Q_t are the maximum amount of pesticides adsorbed (mg/g) and pesticides adsorbed (mg/g) at time t (min) respectively. K_1 (1/h) is the first order rate constant. Therefore

First order rate constant K_1 and Q_{max} were calculated from slope and intercept of plot $\log (Q_{max} - Q_t)$ versus t.

Elovich equation

This equation establishes that the sorption kinetics take place in two phases. A rapid initial stage associated with the movement of the pesticide to the most accessible

parts of the soil, followed by a slower second stage where diffusion particles in soil micropores occur. The linear form of the equation is given as

$$Q_t = 1/Y \ln (XY) + 1/Y \ln. t$$

Where Q_t is the sorbed quantity (mg kg^{-1}) at time t, and X and Y are empirical constants. The intercept ($1/Y \ln (X Y)$) correspond to the sorbed quantity for the first phase and the slope 1/Y, represents the duration of second phase.

Weber - Morris model

This equation establishes that many sorption processes vary proportionally with $t^{1/2}$, where Q_t is the pesticide adsorbed (mg kg^{-1}) at time t, C (mg kg^{-1}) is a constant related to the thickness of the boundary layer and kint (mg $kg^{-1}h^{1/2}$) is the intraparticle diffusion rate constant

2.2.3. Column leaching studies

The column (30-cm length, 6-cm diameter) was made from Poly Vinyl Chloride (PVC) pipe, fitted with 0.60 µm nylon membrane. The soil loss from the column outside was prevented by placing wool in the nylon fiber. To obtain uniform packing, the soil was added to the column in small portions with a spoon and pressed with a plunger under simultaneous gentle column vibration until the top of the soil column did not sink in further. Uniform packing was required for obtaining reproducible results from leaching columns. After packing, the soil columns were pre-wetted with artificial rain (0.01 M $CaCl_2$) applied to the soil surface in order to displace the air in the soil pores. The leachate was collected and their volumes were recorded. Next day the three pesticides chlorpyrifos, dimethoate and quinalphos were added separately to the surface of column and left for 24 hours. The amount of pesticide to be applied to the cylindrical soil column was calculated by the following formula:

$$M(\mu g) = \frac{A(kg/\ ha).10^9(\mu g/kg).\ d^2(cm^2).\pi}{10^8\ (cm^2/ha).4}$$

Where:

M = amount applied per column [µg]

A = rate of application [kg ·ha-1]

d = diameter of soil column [cm]

π = 3.14

The leachate was collected and volume recorded. On the third day again the column was eluted with artificial rain at the flow rate of 100 ml/hour and the lechate was

collected for analysis. After elution was over the soil of column was divided into four sections of 5 cm each to determine adsorbed pesticide on soil.

Three leachates were collected in total (leachate collected after artificial rain-A, leachate collected after the addition of pesticides- B and leachate collected after the addition of final artificial rain-C). 5 ml leachate was mixed with equal volume of chloroform (5ml) and centrifuged for 20 minutes at 30^{0}C at 4000 rpm which resulted in the formation of two layers-the bottom organic layer and top water layer. The bottom chloroform layer was separated using the syringe and transfered to the Eppendorf tube after filtration by micro syringe and anlysed using GCMS.

The soil sample in the column was segmented to 4 layers having 5 cm thickness each and each soil section was mixed well. Weighed about 5 gm soil from each section and mixed it with an equal amount of chloroform (5 ml). The sample - chloroform mixture was centrifuged at 30^{0}C for 20 minutes at 4000 rpm. The bottom chloroform layer containing pesticide residues was collected for analysis in GC - MS. The pesticide concentrations in the sections were added up to give the total pesticide retained by surface/ subsurface soils.

2.3. Field experiments

2.3.1. Layout, design, treatments, sampling frequency and analysis

In order to carry out analysis of *in situ* pesticide residue transfer and degradation, a field experimental set up was established at the campus of Cardamom Research Station, Kerala Agricultural University, Pampadumpara. The area is located at the heart of Cardamom Hill Reserves and represents the actual field conditions prevailing in cardamom growing areas.

The pesticide treatment groups were set up as per following details

Pesticides	:				
Dimethoate	-OP	- Systemic	- 0.05 %	1 lit/clump	- Spray
Chlorpyrifos	-OP	- Contact	- 0.04 %	2 lit/clump	- Drenching
Quinalphos	-OP	- Contact	- 0.05 %	1 lit/clump	- Spray

Pesticide application

Every thirty days – Starting from 1st day of application for a period of one year. Dimethoate and quinalphos were applied monthly and chlorpyrifos was applied from September to October and May to June.

Doses

The pesticide doses were fixed based on the package of practice (POP) for cardamom, recommended by Kerala Agricultural University. A total of three doses and a control was set up. Control (A), ½ x POP recommended dose (B), POP recommended dose (C) and 2 x recommended dose (D). Cardamom saplings were planted at a distance of 3 m apart. Destructive sampling was done on every third month for a period of one year.

2.3.1. Transfer factors

The entry of trace contaminants, which are present in the terrestrial environment, into human food chain is controlled in the long term by their uptake by plant roots. For pesticides, it is generally assumed that the concentration of a moiety in a plant or plant part, C_i^P (mg kg^{-1} , dry weight), is linearly related to its concentration in soil within the rooting zone, C_i^S (mg kg^{-1}, dry weight),

i.e. $C_i^P = TF \times C_i^S$

The proportionality constant TF is called the soil-to-plant transfer factor (or concentration ratio) and is given as

$$TF = \frac{\text{concentration (mg per kg) dry plant tissue}}{\text{concentration (mg per kg) dry soil}}$$

3. Results and Discussion

3.1. General soil characters in Cardamom Hill Reserves

The soil reaction in CHR varied between pH 3.56 and 6.98 in all the analyzed soil samples. Nearly 65 % of the samples were very strongly to extremely acidic (Figure - 4.3). The soils of Kerala in general are acid in reaction brought out by the intense rainfall and consequent leaching of bases. The soil acidity of CHR is aggravated by heavy input of acidic fertilizers.

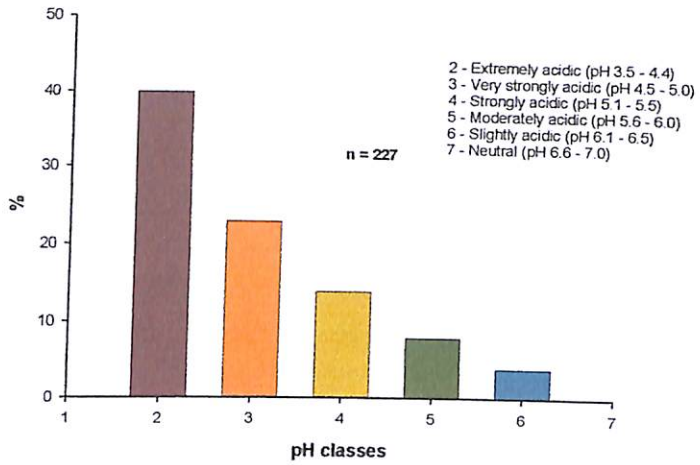


Figure - 4.3 Frequency of soil reaction classes of CHR

Soil organic carbon content (Figure - 4.4), was found to be very high in most of the soils of CHR. The low temperatures in the region restricts decomposition and loss of organic matter. Besides, most of the cropping systems in CHR are managed with huge additions of organic manures which also contributes to the organic matter pool. The partial decomposition may however have a negative impact on soil N contents and C:N ratios in soils. Only less than 10% of the soils were found to be with very low, low or medium organic matter status.

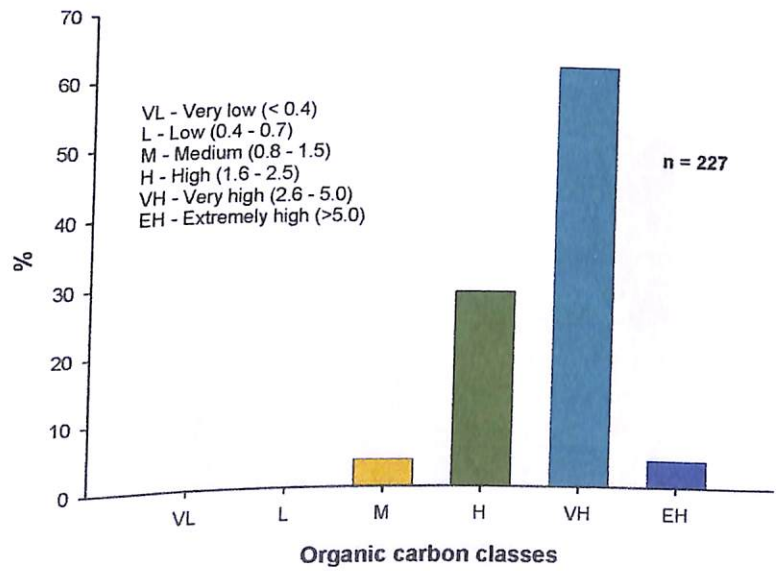


Figure - 4.4. Frequency of soil organic carbon classes of CHR

Plant available phosphorus (Figure - 4.5) was deficient in less than 20 % of the soils in CHR. Generally, soils in the tropical region are with high sesquioxide contents which convert the P into non - soluble Fe-P or Al-P. However, the high organic matter content and high input of phosphatic fertilizers over a period of time have ensured high levels of available P in 80% of the soils in the region. Manure application guidelines are frequently based on the N requirements of crops, and P is therefore often oversupplied and liable to accumulate in soils. The short-term phosphorous availability to crops is mainly influenced by biochemical processes that affect organic matter, while geochemical transformations determine its long-term status. The effects of manure on P availability in various soils has been widely studied, and the general conclusion has been that it is a source of P; interacts with soil components in a manner that increases P recovery by crops; and enhances the effectiveness of inorganic P fertilizer. P added from manure and other sources, however, tends to become less available to plants with the passing of time (Sample et al, 1980). The affinity constants and sorption capacities of soils for P are reduced by organic amendments, especially manure. This can be due to competition for P fixation sites by organic acids, and/or the complex formation of exchangeable Al and Fe by components of manure. The latter may, at least partially, be ascribed to the release of sulfates and fluorides by the manure, both of which are strong complexing agents for Al and Fe (Reddy *et al.*, 1980; Iyamuremye, 1996).This points to the need for either skipping or minimizing the use of costly phosphatic fertilizers in the region.

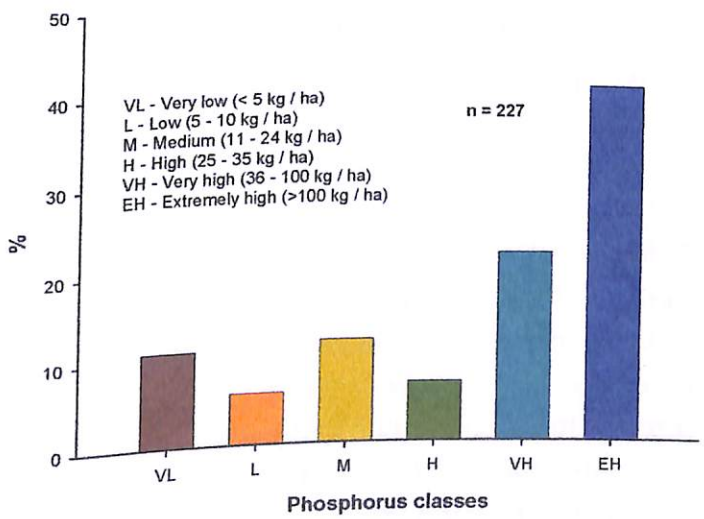


Figure - 4.5. Frequency of available soil phosphorus classes of CHR

More than 90% of the analyzed samples were found to be adequate or high in available potassium (Figure 4.6). The potassium content in soils ranged from 52 - 2200 kg/ ha. In soils, clay and organic matter particles hold potassium ions in an exchangeable or available form. Some leaching may take place in coarse textured soils because such soils do not contain enough clay to hold the potassium. In soils of CHR, as the clay content is low, organic matter would be holding most of the positively charged nutrients tightly. Potassium is an exception because the attraction between potassium ions and organic matter particles is relatively weak. Consequently, potassium leaching in these soils will be very high and presence of high potassium content indicates heavy potassium fertilization in the region.

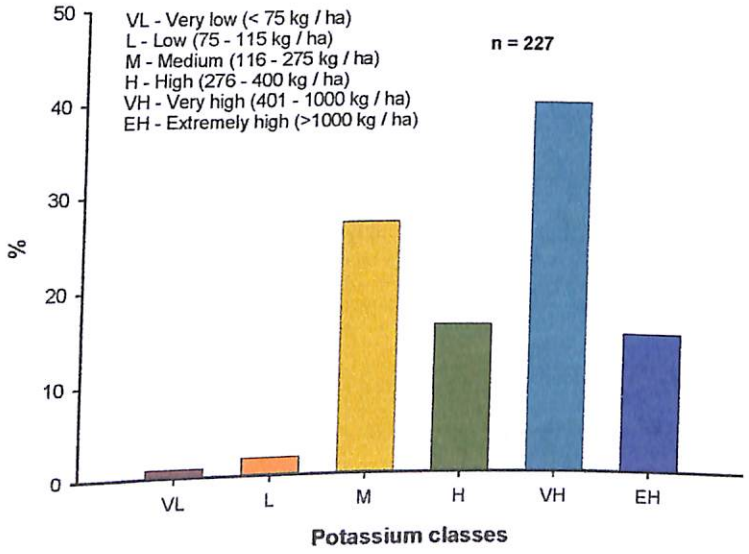


Figure - 4.6. Frequency of available potassium classes of CHR

3.2. Laboratory experiments

Sorption and its kinetics are important processes affecting the leaching of pesticides through soil because it controls the amount of pesticide retained and made available for transport. Physicochemical properties of soil can significantly affect pesticide transport and the potential for groundwater contamination. These properties include soil texture, organic matter, pH etc. The sorption and kinetic studies were conducted using soils collected from Cardamom Research Station, Pampadumpara experimental soils were strongly acidic, medium in organic carbon, low in nitrogen and phosphorus. Potassium, calcium and magnesium were found to be high in these

soils. The study site at was found to have 630 μg kg^{-1} of Microbial Biomass Carbon (MBC) (Table - 4.1).

Table - 4.1. General characters of the experimental soil

Soil Parameters	Values
Sand (%)	82.0
Silt (%)	10.0
Clay (%)	8.0
pH	5.4
Organic Carbon (%)	2.4
N (kg ha^{-1})	474.3
P (kg ha^{-1})	1.8
K(kg ha^{-1})	405.9
Calcium (mg kg^{-1} soil)	379.9
Magnesium (mg kg^{-1} soil)	327.5
MBC (mg kg^{-1} soil)	630.0

3.2.1. Sorption characteristics

The effect of soil pH on pesticide adsorption when different pesticides were added @ 40 mg/ kg soil is given in Table - 4.2. No significant difference was found in pesticide retention when the soil pH was reduced from 5.4 to 3.5. On an average 99.9% of the pesticides were removed by way of adsorption on to the soil matrix at pH = 5.4. On decreasing the pH, there was 100% adsorption in all the cases. Adsorption of pesticides in soil reduces the pesticide's reaction effectiveness. Adsorption and desorption of a pesticide in soil is influenced by the: a) physicochemical nature of the adsorbent (ie., pesticide); b) physico-chemical nature of the adsorbate (ie., clay); c) electrical potential of clay surfaces; d) temperature; and e) soil pH. Earlier works also establishes that adsorption of organic chemicals to soil colloids increases as soil pH decreases (Adams, 1972; Adams and Pritchard, 1977). The experiments reveals that the soils have good adsorption but the adsorptive forces which binds the pesticide on to the soil colloids should be strong enough to immobilize the adsorbed ion or particle. The type of adsorption and the bonding strengths by way of adsorption isotherms and thermodynamic parameters discussed in the following sections.

Table - 4.2. Effect of pH on pesticide adsorption (%) on the studied soil

Pesticide	pH	Adsorption (%)
Dimethoate	3.5	100
	5.4	99.9
Chlorpyrifos	3.5	100
	5.4	99.9
Quinalphos	3.5	100
	5.4	99.9

3.2.2. Adsorption isotherms

The adsorption behavior of pesticides (chlorpyrifos, dimethoate and quinalphos) on the experimental soils were evaluated by Langmuir and Freundlich's adsorption isotherm models. The Langmuir isotherm assumes monolayer adsorption onto a surface containing a finite number of adsorption sites of uniform strategies with no transmigration. The constants k and Qmax in the model relate to the energy of adsorption and maximum adsorption capacity respectively. A perusal of the fit values shows that the Langmuir model could describe the pesticide adsorption only weakly and that too at higher pH values (Table 4.3). The heterogeneity of soil system prevents a linear Langmuir fit and only approximations for the fit parameters could be derived. The non linearity of the isotherms can be due to lower hydrophobicity of the studied pesticides and therefore not limited by solubility at extremely low concentrations (Metwally et al., 2008; Mohapatra et al., 2009; Srivastava et al., 2007; Çelekli et al., 2011). Moreover there can be at least three time scales describing a monolayer adsorption kinetics onto a surface containing a finite number of sites.

Table - 4.3. Langmuir isotherm parameters for adsorption of pesticides onto soil colloids at different pH.

Pesticides	pH	Qmax (mg/kg soil)	k (L/ mg)	R^2
Dimethoate	3.5	6.55	15.00	0.96
	5.4	24.74	6.00	0.54
Chlorpyrifos	3.5	44.83	42.00	0.42
	5.4	43.27	51.00	0.59
Quinalphos	3.5	8.61	66.00	0. 21
	5.4	24.42	3.00	0.90

1. Minutes: there is a rapid, reversible diffusion and adhesion to "accessible" sites of soil surfaces near the soil/water interface.

2. Hours to days: a slower exchange of pesticide between water and/or the labile sites and more slowly-exchanging soil sites occurs. This exchange appears to be fully reversible.

3. Weeks to years: a very slow reaction, which is generally referred to now as "*ageing*". Ageing stores intact pesticide molecules, which may be freed by subsequent processes.

The capturing of all these processes in a single experiment with limits on time produces its own complexities. The model values makes it clear that there is more than one interaction site on the soil surfaces. The process of interaction of the pesticide molecules with the soil usually involves more than one mechanism although a single mechanism may dominate, depending on the physical and chemical conditions of the soil, its mineral composition, adsorption conditions and concentration of pesticides. All the pesticides showed a higher adsorption at pH 5.4. At pH 5.4, the strengths of retention of pesticides varied as chlorpyrifos > dimethoate > quinalphos. As the pH was dropped from 5.4 to 3.5 the pesticide adsorption decreased dramatically but was retained with more strength as indicated by the b values. Chlorpyrifos showed the highest *Qmax* at both pH and the values

were comparable showing that its adsorption doesn't change much with pH in the studied soil system. For dimethoate and quinalphos, Langmuir parameters, *Qmax and k*, increased with decreasing pH. The bonding strength values for dimethoate increased by 2.5 times and that of quinalphos by 22 times as the pH decreased from 5.4 to 3.5. However the corresponding values for *Qmax* decreased by 73% and 64% for dimethoate and quinalphos, respectively. pH effects on b values indicate that these pesticides are more strongly adsorbed to the mineral surfaces as acidity increases but the amount retained decrease (Sparks, 2003).

As the soil reaction is reduced, the loss of clay crystallinity and organic matter charge reversal may severely affect the amount of organic molecules retained. However at pH 3.5, more transitional metal ions are available as a result of acid dissolution of free metal ions which chelate some of the pesticide molecules, hence higher b values at lower soil reaction. This can have important implications during acidic fertilizer application in which agricultural operations contribute H^+ ions to soil.

A comparison of the linear plots of specific adsorption against the equilibrium concentration for all the studied pesticides shows that the adsorption obeys more of the Freundlichs equilibrium isotherm than Langmuir model (as indicated by their R^2 values). Hence the Freundlich isotherm may be considered a more suited model to describe sorption kinetics of pesticides in the experimental soils. In Freundlich's isotherm, K_f may be considered as an index of pesticide adsorbed (higher k = higher adsorption) from solutions having a unit equilibrium concentration and $1/n$ indicates intensity of adsorption. The $1/n$ value indicates the degree of nonlinearity between solution concentration and adsorption as follows: if $1/n = 1$, then adsorption is linear; if $1/n > 1$, then adsorption is a chemical process; if $1/n < 1$, then adsorption is a physical process. Linear relationships between ln x/m and ln Ce was obtained for dimethoate and chlorpyrifos at pH 5.4. Figure - 4.7 to 4.9 shows that marginal increase (unit adsorption per unit increase in concentration) in adsorption decreases with decrease in soil pH for dimethoate and chlorpyrifos. Dimethoate is retained by chemisorption at pH 5.4 but is held by physisorption at lower soil reactions (Table - 4.4). Quinalphos had a reverse trend whereas chlorpyrifos was chemisorbed at all pH values. In short, dimethoate at higher pH, quinalphos at lower pH and chlorpyrifos at all pH are retained by chemical bonding.

Table - 4.4. Freundlich isotherm parameters for adsorption of pesticides onto soil colloids at different pH.

Pesticides	pH	1/n	K_f	R^2
Dimethoate	3.5	0.65	0.52	0.86
	5.4	1.12	2.03	0.96
Chlorpyrifos	3.5	1.53	3.49	0.93
	5.4	1.54	3.40	0.96
Quinalphos	3.5	2.04	5.20	0.60
	5.4	0.50	0.06	0.86

Figure - 4.7. Freundlich isotherm of dimethoate adsorption on CHR soils at pH 5.4 and 3.5

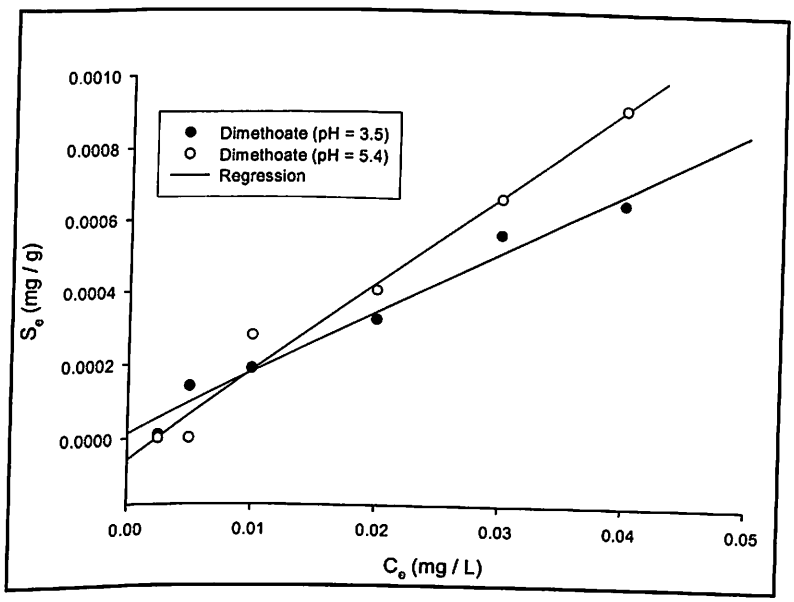


Figure - 4.8. Freundlich isotherm of chlorpyrifos adsorption on CHR soils at pH 5.4 and 3.5

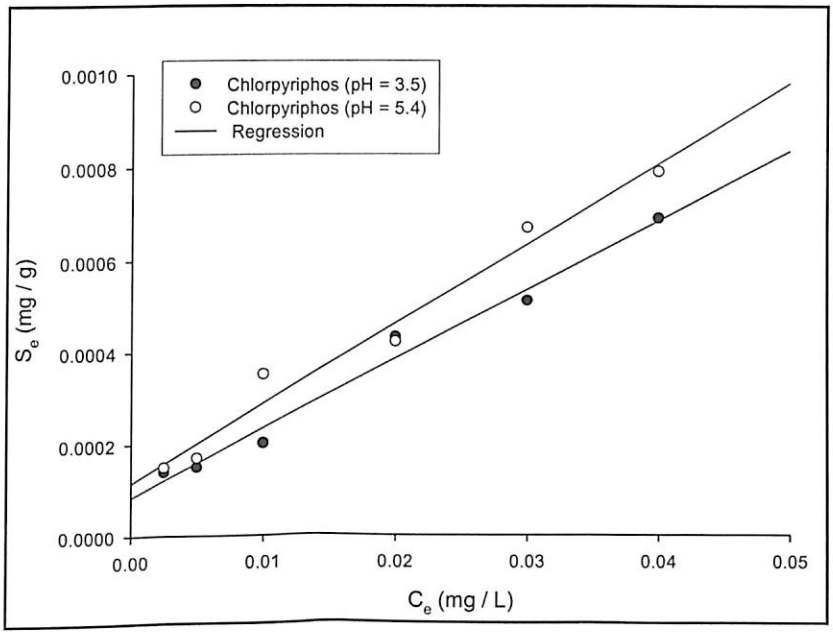
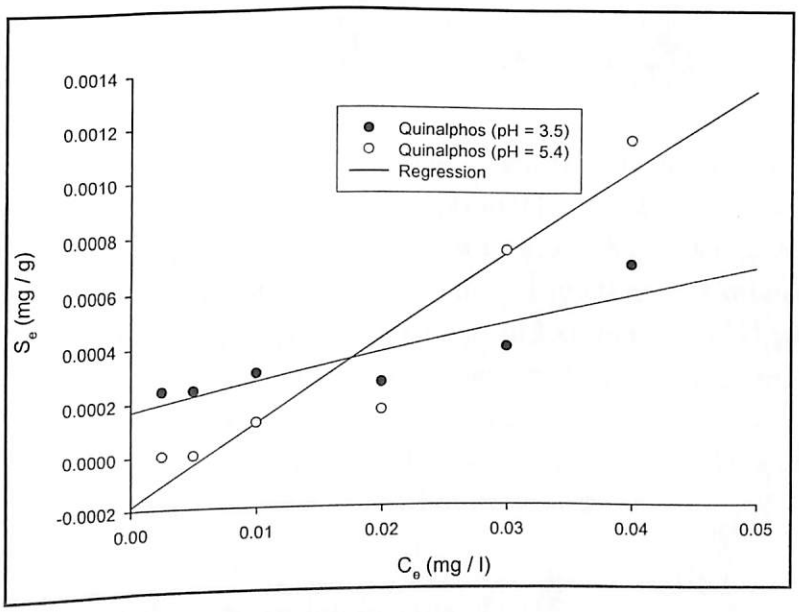


Figure - 4.9. Freundlich isotherm of quinalphos adsorption on CHR soils at pH 5.4 and 3.5



Thermodynamic considerations of an adsorption process are necessary to conclude whether the process is spontaneous or not. Gibb's free energy change, $\Delta G°$, is the fundamental criterion of spontaneity. Reactions occur spontaneously at a given temperature if $\Delta G°$ is a negative value. In the present study, Gibb's free energy (ΔG^0) values were found to be negative in all the adsorption cases (Table - 4.5). The values varied from -16 to -20 kJ/mol at pH 5.4 and -21 to -23 kJ/mol at pH 3.5. The negative value of $\Delta G°$ indicates that the adsorption process of pesticides in soil is a spontaneous process (Boparai et al., 2011;Wu et al., 2007).

Table 4.5. Variation in Gibb's free energy (ΔG^0) of dimethoate, chlorpyrifos and quinalphos with soil pH

Pesticide	pH	ΔG^0 (kJ/mol)
Dimethoate	3.5	-23.2
	5.4	-16.8
Chlorpyrifos	3.5	-21.9
	5.4	-20.7
Quinalphos	3.5	-23.2
	5.4	-19.7

Based on the adsorption and thermodynamic studies it can be concluded that Freundlichs isotherm best explains the sorption kinetics of pesticides in soils of CHR. Among the different pesticides used in the study, the soils showed maximum adsorption potential for chlorpyrifos followed by dimethoate ≈ quinalphos at pH 5.4. From the adsorption isotherms it can be concluded that the adsorption capacity of soils for pesticides decreases with soil reaction but the binding strength increases. The lesser retention with decrease in pH poses a threat that uncontrolled use of pesticides coupled with decrease in soil pH may lead to huge leaching of the applied pesticides to ground water/ water bodies from these soils.

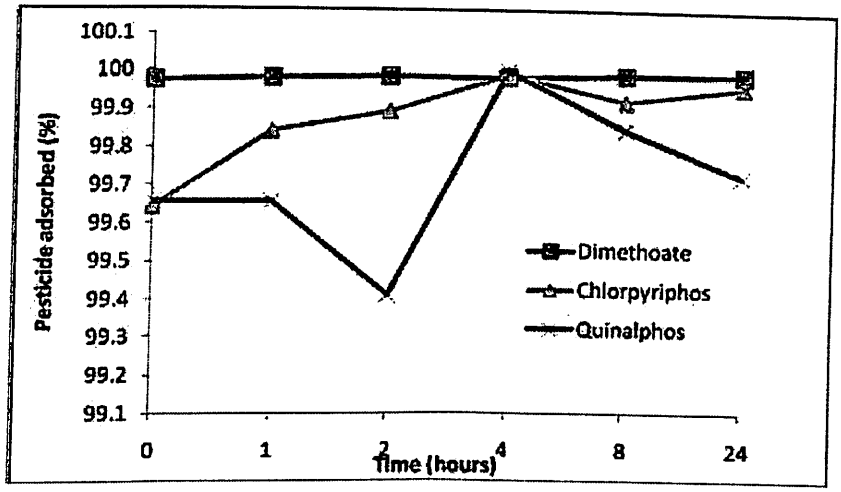
3.2.3. Kinetics of pesticide adsorption in soils of CHR

Kinetic adsorption reveals the removal rate of solute which controls the residence time of the pesticide in the solid - solution interface. The kinetic adsorption for the

three commonly used pesticides - dimethoate, chlorpyrifos and quinalphos is shown in Figure - 4.10. The sorption of pesticides on to soil colloids rapidly increased upto 4 hours of shaking. More than 99% adsorption was noticed for all the studied pesticides. Dimethoate and chlorpyrifos were found to be retained on the soil colloids without much desorption throughout the experiment. Quinalphos on the other hand showed a gradual desorption with time. As discussed earlier the bonding strengths of these two pesticides are greater than quinalphos at pH 5.4, the experimental soil pH. The variable charged minerals and pH dependent charge sites on organic matter resulted in a fluctuating adsorption pattern during the initial phases (upto 4 hours). The pesticides were adsorbed rapidly during first stage due to the vacant sites available in soil initially. As the exchange complex gets saturated, the concentration gradient between solid and solution gets reversed which causes the slow migration and diffusion of pesticide molecules towards the soil matrix during the second phase. From the studies it can be concluded that overloading of pesticides beyond the maximum retaining capacity (> Qmax values at a given pH) may lead to increased pesticide leaching in these soils. The time taken for the adsorbed pesticides to degrade should also be considered when pesticide spray schedules are planned.

The soils of the study site were extremely acidic in reaction. In acidic pH, the studied pesticides are partially in a cationic form due to protonation of amino groups and thereby get easily adsorbed to the negatively charged colloidal surfaces. On the other hand, it is know that the pH in the soil surface may be lower than in the soil solution, meaning that a higher number of neutral pesticide molecules could also be adsorbed. On the surface of organic matter the pH might be 0.2 - 0.5 units lower than liquid phase thereby retaining large quantities of pesticide moieties. Different kinetic models were applied to the experimental data in order to determine the kinetic parameters and to have further information on the adsorption mechanisms involved.

Figure - 4.10. Sorption kinetics of pesticides in soils of CHR



The pseudo - first order model didn't show a good fit with the experimental data for chlorpyrifos and quinalphos, with R^2 values <0.50. The maximum adsorption values obtained were also not consistent with experimental values (Table - 4.6). Out of the three pesticides, quinalphos showed the lowest kinetic rate constant (K_1) indicating very slow adsorption of this pesticide on to the soil matrix. This low K_1 coupled with low b values (Langmuir values) causes majority of the pesticide reaching the soil to remain in an unadsorbed state for long time periods. In Elovich model, used to describe the pseudo - second order kinetics, the intercept $(1/Y)$ ln (XY) represents the amount adsorbed during the initial equilibrium phase (fast phase reaction) and the adsorption rate as a function of time during the slow phase of the reaction respectively (Cáceres et al., 2010). The results indicate that 99 % of the total pesticide sorbed is during the fast phase pointing to an almost instantaneous adsorption equilibrium for all the studied pesticides. However, the time required for the fast phase may vary among the pesticides and if read along with pseudo – first order results it would be safe to assume that dimethoate and chlorpyrifos adsorptions occur within a short time after application as compared to quinalphos.

The Weber - Morris model that relates qt versus $t^{1/2}$ generates a straight line that passes through the origin, when the intra particle diffusion process controls the sorption mechanism. C intersect values provide information relating to the thickness of the boundary layer, a larger C value meaning a higher boundary layer effect. Table - 4.6 and Figure - 4.11 to 4.13 show the kinetic parameters obtained for this model. The results show that C values are positive and highest for quinalphos. Larger boundary layer will result in weaker forces of retention. This further

corroborates our earlier results that quinalphos is weakly retained by these soils and hence slowly adsorbed and easily desorbed. In these soils, mass transfer across the boundary layer by way of intra particle diffusion were highest for chlorpyrifos (0.01mg/kg soil/h) followed by quinalphos (0.006 mg/kg soil/h). As for the intra-particle diffusion, the straight line for any of the three pesticides did not pass through the origin, which shows that the rate of these pesticide adsorption onto soil is limited by mass transfer across the boundary layer. A high saturation of the soils for any of these pesticides will thus be determinal in overriding the pesticide retention mechanisms in soil, hence pollute the soil environment. Studies by Cáceres et al., (2010) have also shown that in Ultisols intraparticle diffusion processes was the main factor that defines pesticide sorption in soil.

Figure - 4.11.Weber - Moris Kinetic model for Dimethoate in soils of CHR

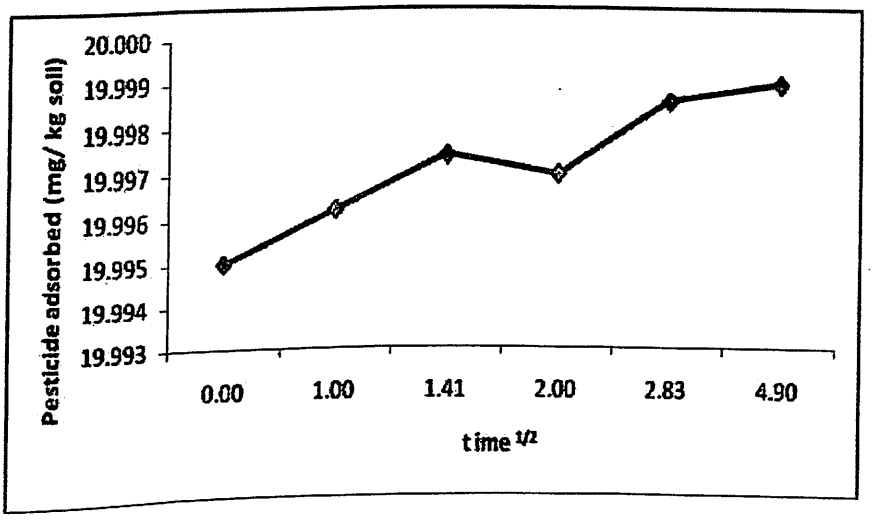


Table 4.6. Kinetic parameters from pseudo - first order, Elovich and Weber - Moris kinetic models of dimethoate, chlorpyrifos and quinalphos in soils of CHR

	Dimethoate	Chlorpyrifos	Quinalphos
Experimental values			
Maximum adsorption (mg/kg soil)	20	20	20
Pseudo First order kinetics			
Qmax (mg/kg soil)	11.53	5.40	2.47
K_1 (h^{-1})	0.06	0.06	0.03
Elovich			
(1/y)lnxy (mg/ kg soil)	19.99	19.950	19.92
1/y (mg/ kg soil)	0.001	0.013	0.01
Weber - Moris			
Kint (mg/kg soil/h)	0.0001	0.01	0.006

Figure - 4.12. Weber - Moris Kinetic model for Chlorpyrifos in soils of CHR

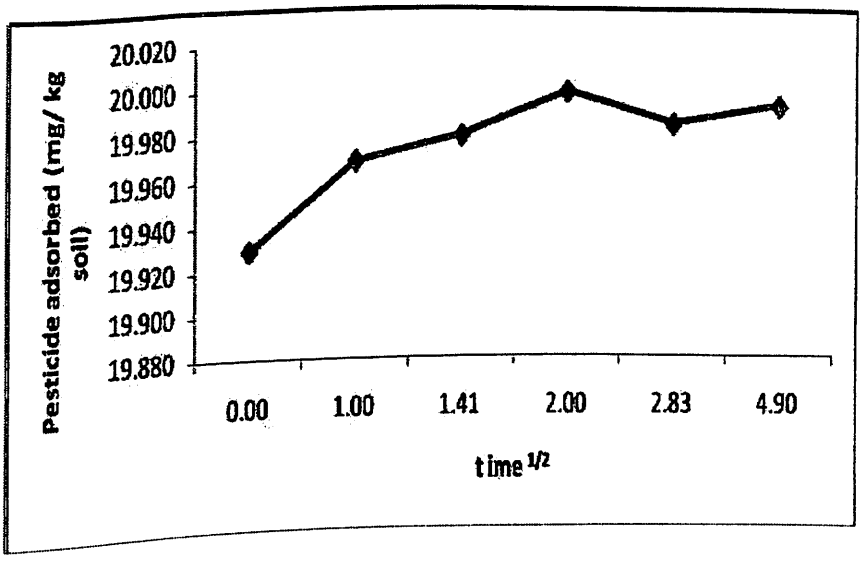
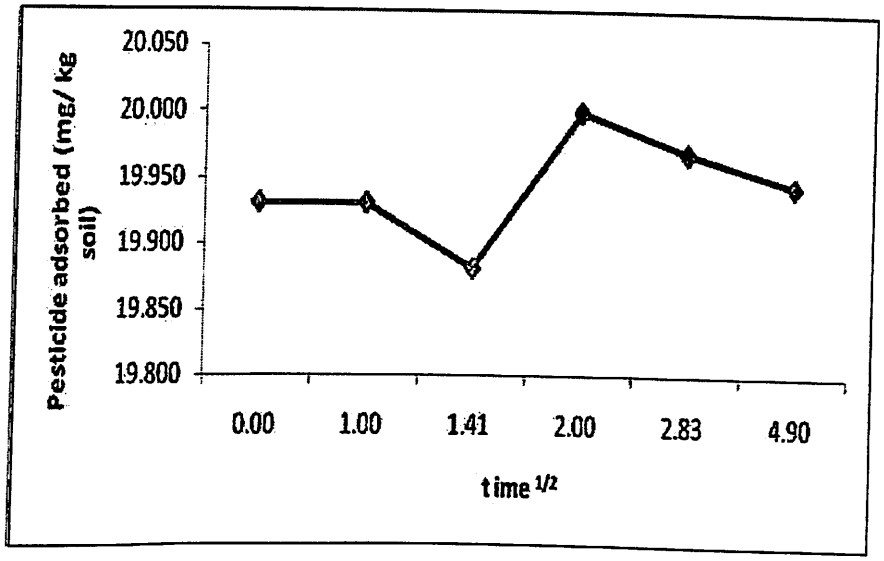


Fig. 4.13. Weber - Moris Kinetic model for Quinalphos in soils of CHR



3.2.4. Column leaching studies

Column leaching experiments following the OECD guidelines were conducted to analyze the leaching potential of these pesticides after they have been applied to soil. Usually the pesticides are more adsorbed to soil having higher clay and organic matter. Even though the study soil contains less clay (8 %), these soils had very high organic matter contents which could provide sufficient adsorption sites for the pesticides (OC = 2.4%). Usually the tropical humid soils are predominated by low activity kaolin clays with CEC values < 10 cmols (p+) kg^{-1} soil. However the high organic matter ensures that the soils have a high cation exchange capacity and a larger surface area, in spite of these low activity clay minerals. The high CEC enhances adsorption and reduces leaching. Similar observations were also made by Bansal (2010) who noticed that the adsorption was positively correlated with soil organic carbon and cation exchange capacity while negatively correlated with soil pH. As the soils of cardamom plantations are intensively managed with huge additions of acid forming fertilizers there is a potential danger of the soil pH dropping in subsequent years. Organic matter induced surface charges are variable (not fixed) and will change with drops in soil reaction. This in turn can adversely affect the current pesticide adsorption patterns i.e., lesser pesticide amounts retained.

Leaching and adsorption are inversely related to each other. The study shows that though > 90% of the applied pesticides are adsorbed on the soil colloids and under continuous leaching conditions dimethoate, chlorpyrifos and quinalphos are removed to the tune of 59.63%, 36.60% and 95.43% respectively (Table 4.7). During the initial stages the amount of pesticides leached was found to be < 2% which increased by 15–40 times by third leaching. Chlorpyrifos with strong chemisorptions and high energy of binding was found to be the minimum leached pesticide in these soils. On continuous application of leachate, bases will be leached out leading to a decrease in soil pH. As discussed earlier, the adsorption in the studied soils are essentially organic matter driven. Decrease in soil reaction will alter the variable charges on these organic colloids and reduce the binding strength leading to increased desorption. The successive raises in desorbed pesticide content can thus be explained by a combined effect of base leaching and changes in organic matter induced exchange sites.

In the experiments, the leaching solutions were applied after twenty four hours during which time the pesticide adsorption would have reached the slow phase (Figure - 4.11 to 4.13). The unabsorbed pesticide molecules left over after the initial fast phase are leached down during the first extraction (< 2%). This leaching disturbs the dynamic equilibrium that has been established between the stationary and bulk solution phases (diffuse double layer) and establishes a concentration gradient. The desorption of the molecules along this gradient brings in large quantities of free pesticide molecules to be subsequently removed by the added solution. Quinalphos which had a larger boundary layer would have been the most weakly retained pesticide in the matrix and hence removed with much ease and quantity as compared to dimethoate and chlorpyrifos. These observations point to the fact that immediate heavy rains/irrigation after pesticide application in these areas will leach most of the applied pesticides to ground water or water bodies thereby polluting them.

Table - 4.7. Pesticides removed in successive stages of leaching from surface soils of CHR

Stages	Pesticides removed (%)		
	Dimethoate	Chlorpyrifos	Quinalphos
Leaching 1	1.24	--	1.74
Leaching 2	1.50	--	2.03
Leaching 3	56.88	36.6	91.67
Total	59.63	36.60	95.43

3.2.5. Degradation of Pesticides

The rate of pesticide degradation was assessed with respect to dimethoate, chlorpyrifos and quinalphos in the soils collected from CRS, pampadumpara. This was intended to give an idea about the time taken by the pesticides to decompose in these soils after application.

Dimethoate

More than 75% degradation of dimethoate was found by 40 days of incubation. The dimethoate decomposition shows two distinct phases. Dimethoate concentration was found constant during the initial stages. However with time there was a decomposition of organic matter and raise in pH. The decrease in organic matter may be due to increased microbial activity. Microbes use carbon in organic matter as a source of their energy and multiply rapidly. Increase in pH towards the neutral range also favours microbial activity (Figure - 4.14 to 4.16). These combined effects leads to rapid dimethoate decomposition during the second phase (steep downward slope).

Fig. 4.14. Degradation of dimethoate with time

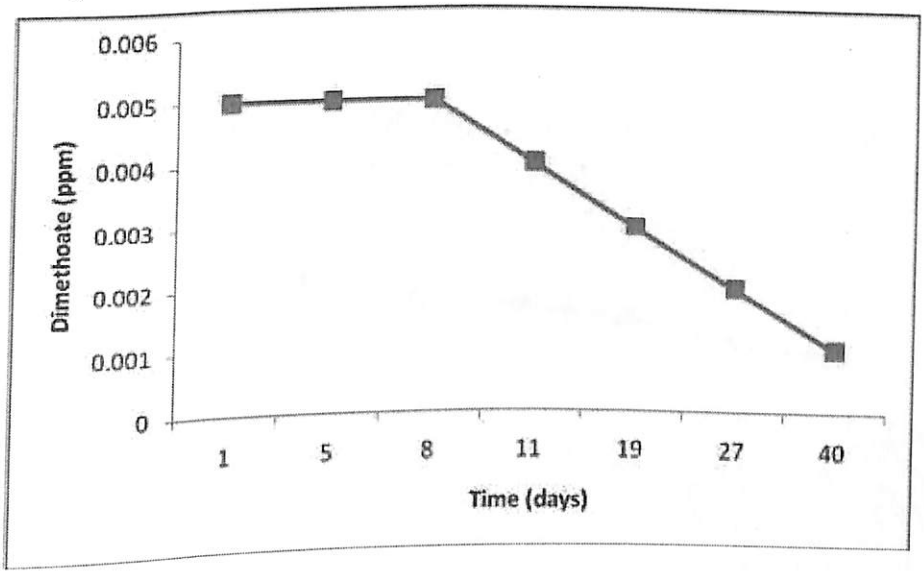


Fig. 4.15. Changes in soil pH during the incubation with dimethoate

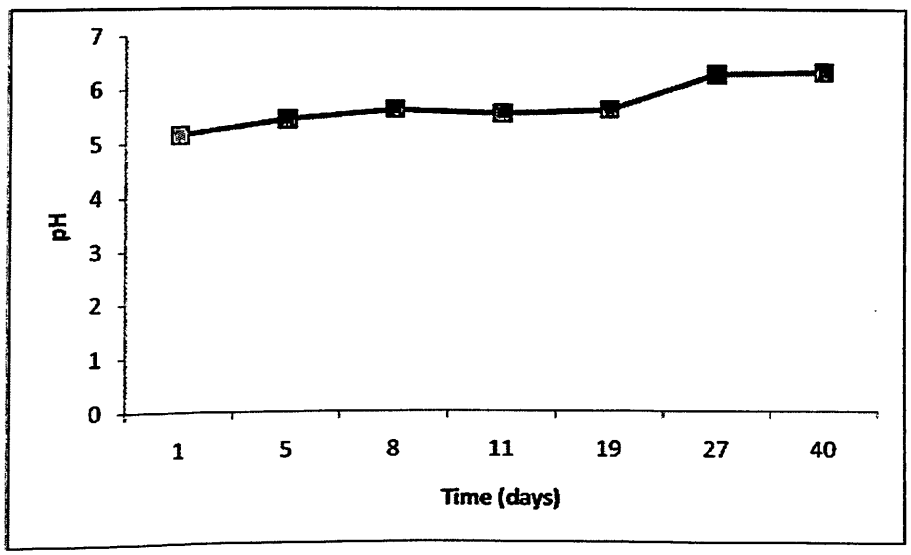
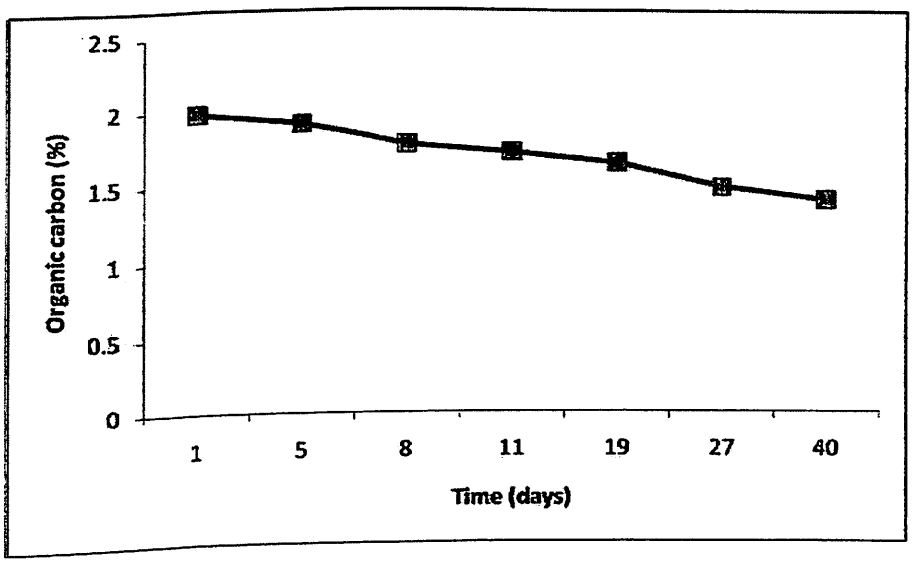


Fig 4.16. Changes in organic carbon (%) during incubation with dimethoate



Chlorpyrifos

Chlorpyrifos was found to degrade more than 90% during the experiment period. Similar to dimethoate, the decomposition of chlorpyrifos was also characterized by two phases - a slow phase and a rapid phase. These two phases were found to correlate with the decomposition phases of the pesticide (Figure - 4.17 to 4.19).

Fig. 4.17. Degradation of chlorpyrifos with time

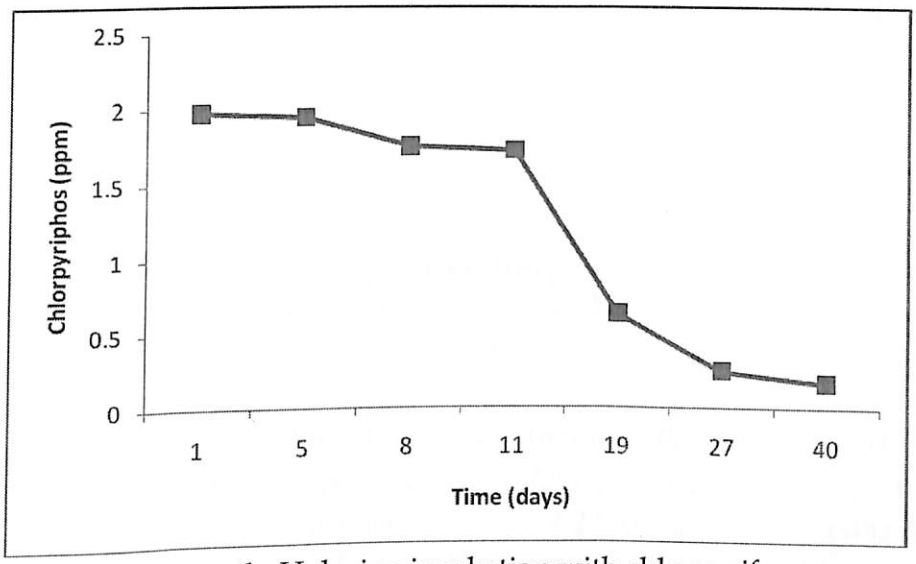


Figure - 4.18. Changes in soil pH during incubation with chlorpyrifos

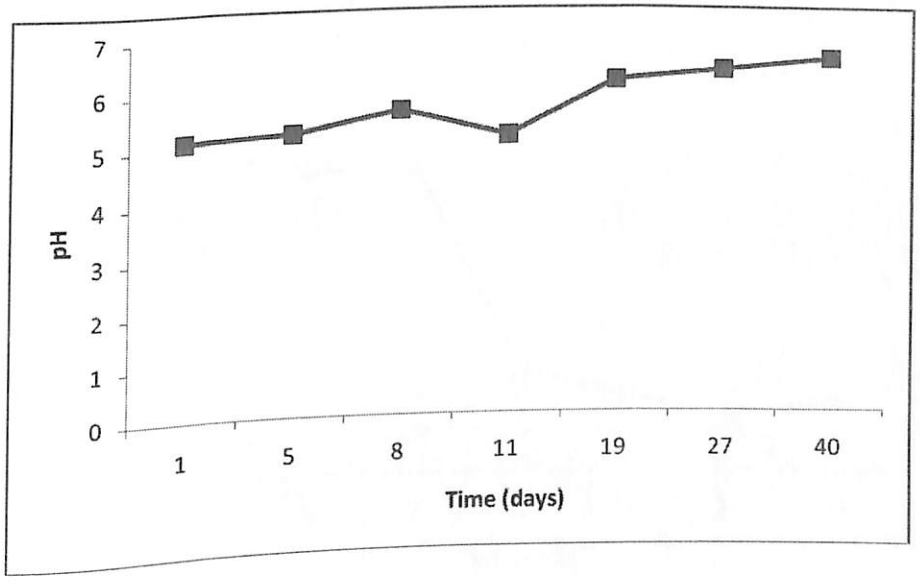
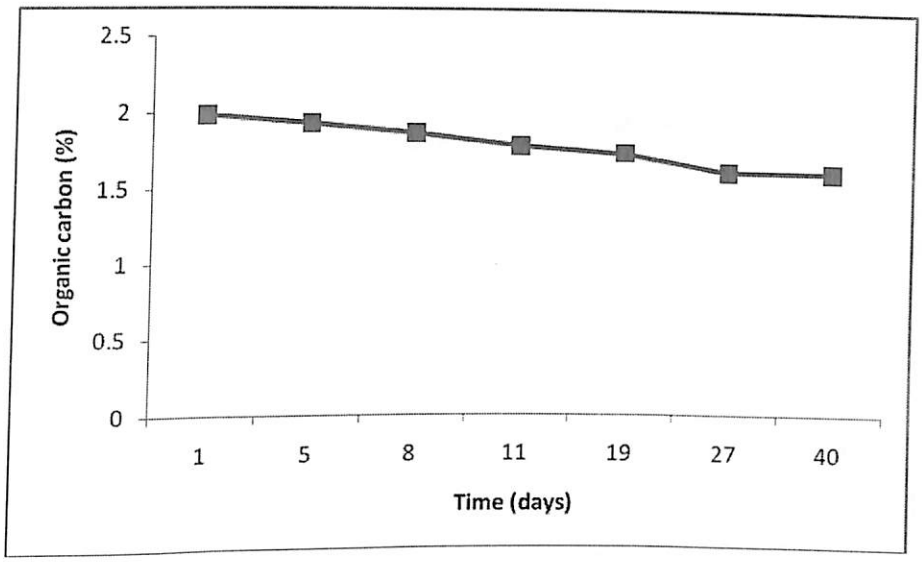


Figure - 4.19. Changes in organic carbon (%) during incubation with chlorpyrifos



Quinalphos

Quinalphos decomposition followed a similar trend as that of chlorpyrifos and dimethoate. All the three studied pesticides were found to be degraded only after an initial restricted slow degradation phase (Figure - 4.20 to 4.22). Heavy rains during this slow degradation period may lead to the washing of these pesticides to nearby water bodies or ground water. Hence care should be taken to avoid the rainy season for the application of these pesticides.

Figure - 4.20. Degradation of quinalphos with time

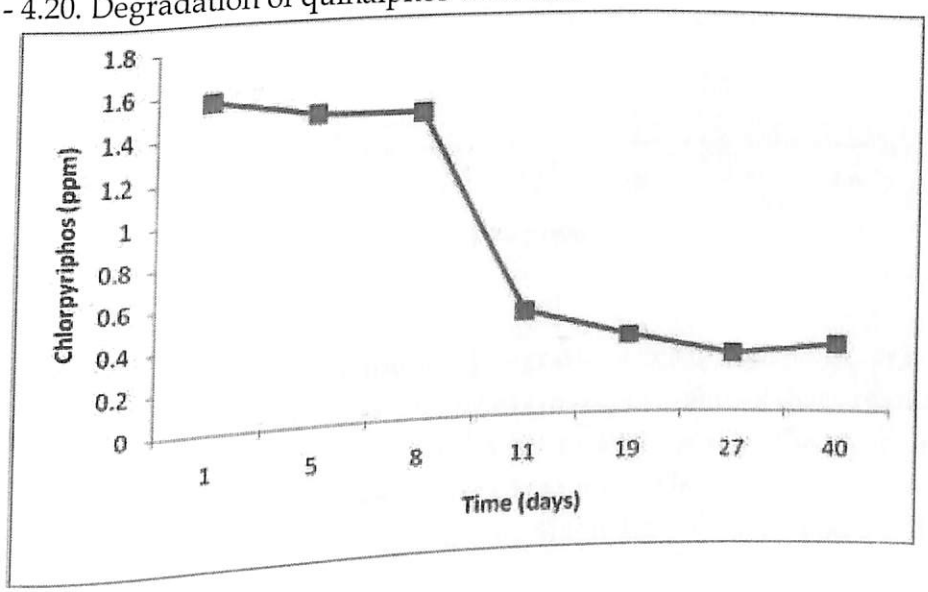


Figure - 4.21. Changes in soil pH during incubation with quinalphos

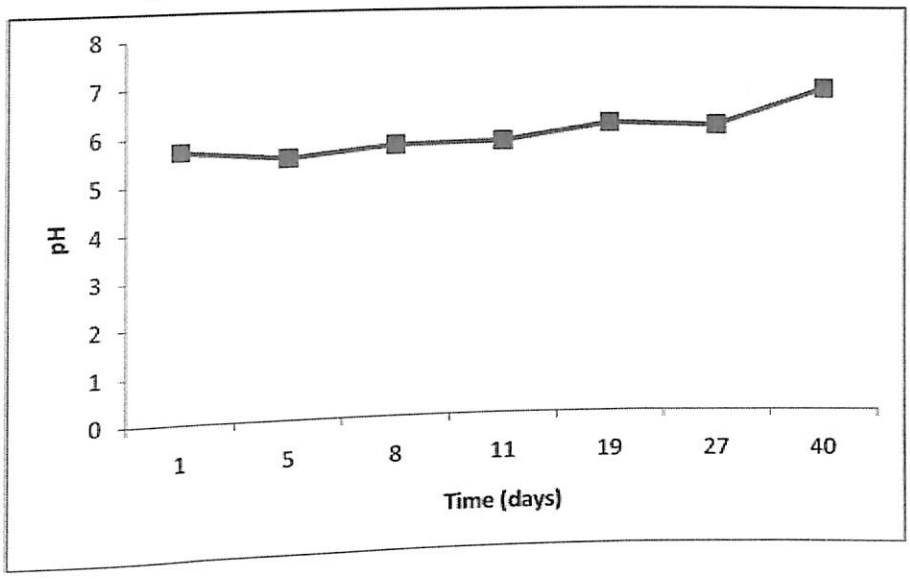
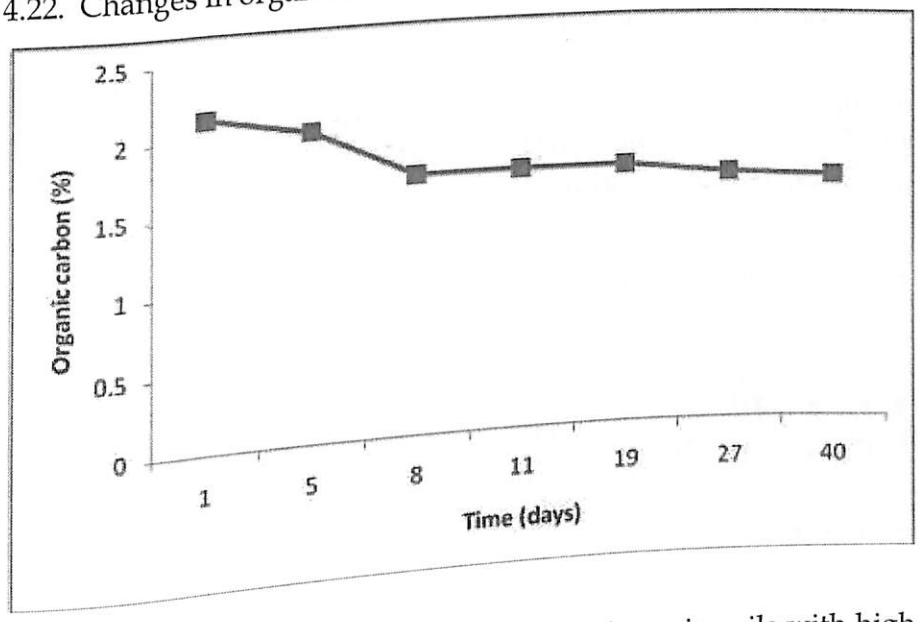


Figure- 4.22. Changes in organic carbon (%) during incubation with quinalphos



Organochlorine insecticides were found to degrade faster in soils with high organic matter content (Castro and Yoshida 1971). Several reports shows that organic matter incorporation, which increases microbial activity and hastens the drop in redox potential in flooded soils, favors pesticide degradation. This was observed for straw incorporation (Adhya et al. 1981; Chopra and Magu 1986; Gowda and Sethunathan

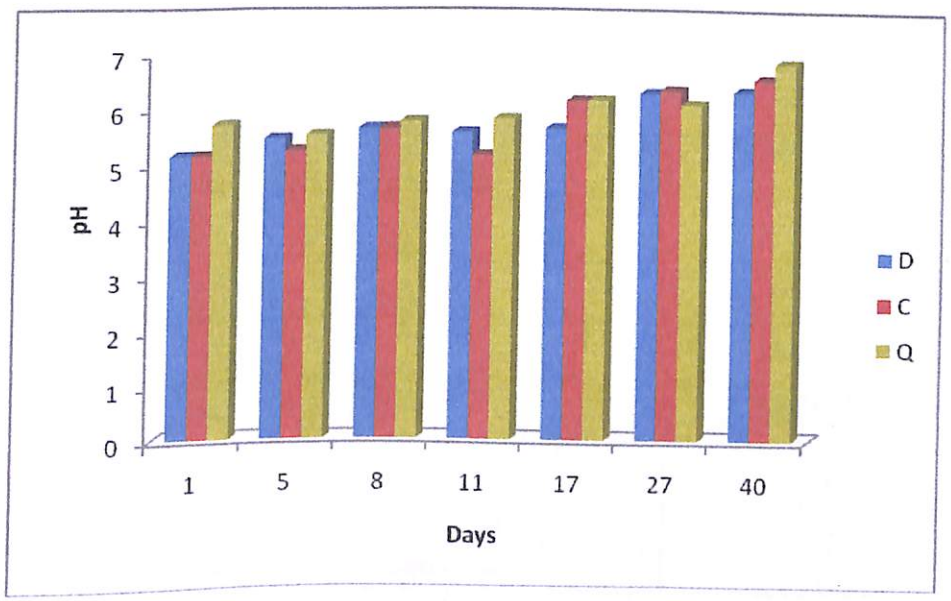
1976; Venkateswarlu & Sethunathan N 1979) as well as for green manure incorporation (Ferreria and Raghu 1981). However, Castro and Yoshida (1971) observed this effect only when soil organic matter was low. The experimental soils with high organic matter contents had a slow initial phase which can be attributed to the restrictions in the carbon to nitrogen balances in them. The microbial cells have a carbon to nitrogen ratio of 10 - 15:1 i.e., for every 10 units of C the cells require one unit of N. Carbon acts as the energy source and N as the building blocks (protein formation) in the growth process of microbial cells. Hence for microbial activity to proceed unhindered we require a C:N ratio of 10 - 15:1. The study soils had a very high C:N value of 120:1. This high value may be due to the low temperatures at these altitudes which restrict decomposition. Hence the initial microbial proliferation and their activities would have been restricted. However, with time the C:N ratio would have reached the optimum limits by way of native N mineralization. This would provide an ideal environment for pesticide decomposition by microbes. Soil reaction is another important factor affecting degradation of pesticides. Sethunathan *et al.,* (1982) presented several examples indicating that both alkaline and acidic conditions could enhance the decomposition of specific pesticides or groups of pesticides. They reported that organophosphates and carbamates are more affected by soil pH than organochloride insecticides. In the present study, at the end of the experimental period soil pH was found to approach the neutral range, a favourable situation for microbial proliferation and decomposition (Table 4.8 and Figure - 4.23). However, it should be noted that pH raises occur only when there is no leaching, a condition which didn't occur during incubation. This further supports the view that phases of pesticide application should take into account the rains, adsorption capacity of soils and time required for degradation to prevent pesticide loading and leaching in these soils.

Addition of pesticide and the adaptation of soil microbes to it will have a time lapse. During the initial slow phase the added pesticides will restrict the microbial activities responsible for both organic matter decomposition as well as that of the pesticide. With time the microbes get adapted and multiply faster and lead to the rapid phase of degradation.

Table - 4.8. Changes in soil reaction during incubation during incubation with different pesticides

Days after incubation	Dimethoate	Chloropyrifos	Quinalphos
1	5.15	5.15	5.15
5	5.45	5.23	5.51
8	5.63	5.63	5.75
11	5.55	5.14	5.8
17	5.63	6.14	6.15
27	6.29	6.34	6.09
40	6.34	6.56	6.86

Figure - 4.23. Soil pH changes during pesticide incubation



3.3. Field experiments

The chlorpyrifos transfer factors (TF) for different doses and times of exposure are presented in Table - 4.9. Chlorpyrifos was applied during September, 2013 and may, 2014. The transfer factor values shows that T_4 (2 x Recommended dose) had the highest TF values at all the stages followed by T_3 (Recommended dose). As discussed earlier, chlorpyrifos has a spontaneous chemisorptions immediately after application with > 99% of the pesticides retained in soil colloids. The initial low TFs during October and January may be due to this strong retentions. With time there will be a gradual release and subsequent plant absorption and accumulation of the

pesticide in the plant parts. However by the 12th month there was a drop in the TF values for all the treatments. The drop may be attributed to non absorption of the pesticide by plants from soil beyond a certain age (selective exclusion), dilution of the existing pesticide moieties within the plant body due to increase in biomass and / or degradation of the pesticides within the plant body. The drop in TF of chlorpyrifos even after application of the second split dose in May, 2014 points to such a possibility. Earlier reports show that several factors appear to be of paramount importance in the eventual transfer of a molecule from soil to plants. Such factors include soil type (CEC, specific sorption properties and type of organic and mineral matter) (Frissel et al., 1990; Cremers et al., 1990; Lembrechts, 1993), type of material and its specific interaction with the soil (pesticide adsorption and speciation in the solid phase), level of potential sorption-competitive species in the soil solution and type of plant (species selectivity and growth stage). The multifactor character of the transfer makes it difficult to establish simple relationships between soil-plant attributes and transfer. A simple approach would be in place to identify the key soil factors needed to set up a relative scale of transfer. In addition to the pesticide molecules available in the soil, the CEC of the soil, the solid- liquid distribution coefficient (Kd) and levels of competitive species in the soil solution for the concerned pesticide can also be used for prediction purposes (Absalom et al., 2001).

Table 4.9. Transfer factors of chlorpyrifos from soils of CHR to cardamom

Treatment	Transfer factor
1 month after application (3 MAP)	
T_1 (Control)	0.0
T_2 (0.5 x Recommended dose)	0.4
T_3 (Recommended dose)	3.9
T_4 (2 x Recommended dose)	9.8
4 months after application (6 MAP)	
T_1 (Control)	0.0
T_2 (0.5 x Recommended dose)	5.5
T_3 (Recommended dose)	7.3
T_4 (2 x Recommended dose)	8.4
7 months after application (9 MAP)	
T_1 (Control)	0.0
T_2 (0.5 x Recommended dose)	2.3

T_3 (Recommended dose)	18.4
T_4 (2 x Recommended dose)	62.1
10 months after application (12 MAP)	
T_1 (Control)	0.0
T_2 (0.5 x Recommended dose)	0.0
T_3 (Recommended dose)	0.0
T_4 (2 x Recommended dose)	0.9

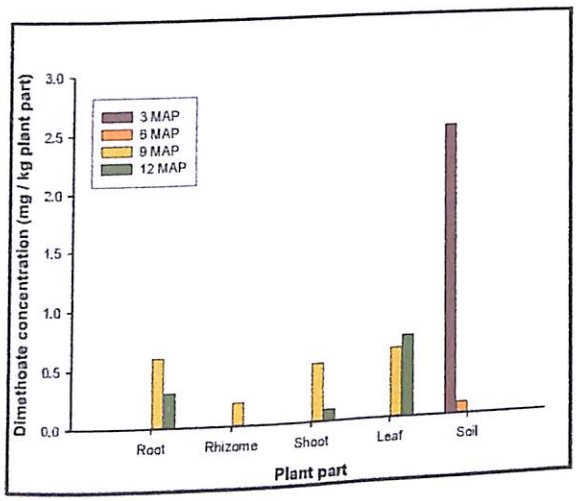
3.3.1. Plant accumulation of applied pesticide

Dimethoate concentrations were found to increase in the plant parts and soil with continuous application. The accumulation was found to increase as 2 x recommended dose (T_4) > recommended dose (T_3) > 0.5 x recommended dose (T_2) (Figure - 4.24 a - c). Lower doses of pesticide (0.5 x recommended dose) will maintain a steady and lower pesticide concentration in the plant body. At lower concentrations, soils were found to have the lowest amounts of pesticide which may be due to leaching or degradation of the applied pesticide in this matrix within a short time. But application of pesticides beyond a critical limit will make the plant body and soils highly concentrated with respect to the pesticides.

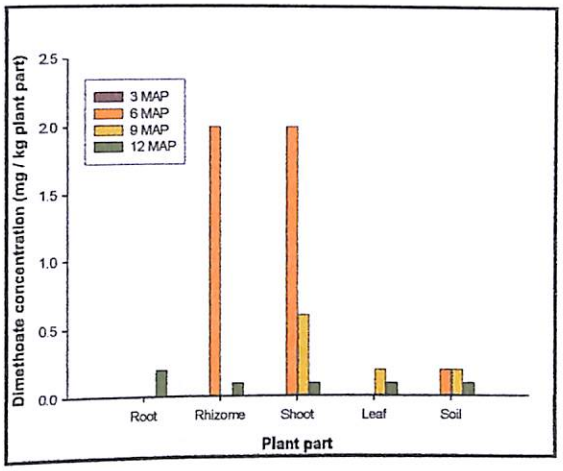
Chlorpyrifos is strongly adsorbed by soils and maximum transfer occurs to the rhizome and roots and above ground vegetative portions show very little accumulation. There was very little variations in accumulation pattern of this pesticide at T_2 and T_4 (Figure - 4.25 a- c). Hence if this particular pesticide can give the desired effects at lower concentrations, this should be recommended. Misuse of pesticides beyond that required dose will only lead to contamination.

Figure - 4.24. Accumulation of dimethoate in plant parts and soil at (a). 0.5 x recommended dose; (b) recommended dose; (c). 2 x recommended dose

a.



b.



c.

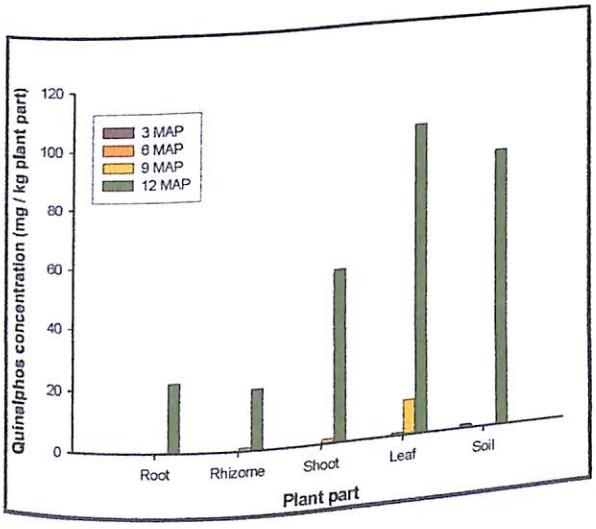
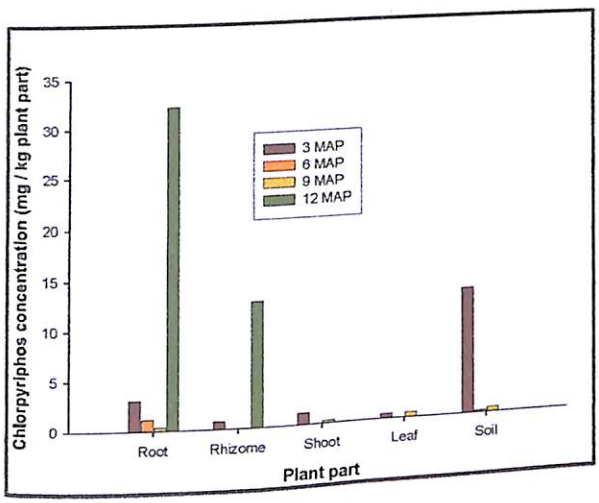
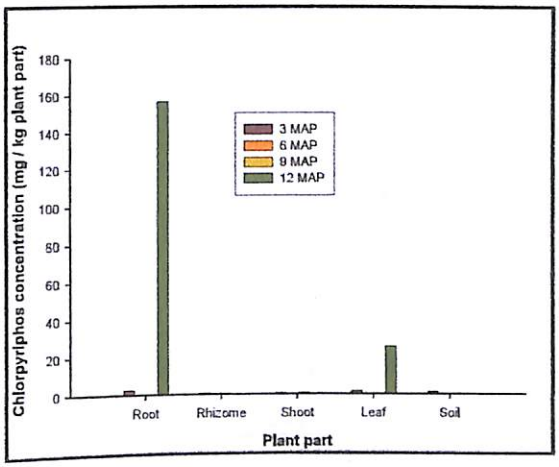


Figure - 4.25. Accumulation of chlorpyrifos in plant parts and soil at (a). 0.5 x rcommended dose; (b). recommended dose; (c). 2 x recommended dose

a.



b.



c.

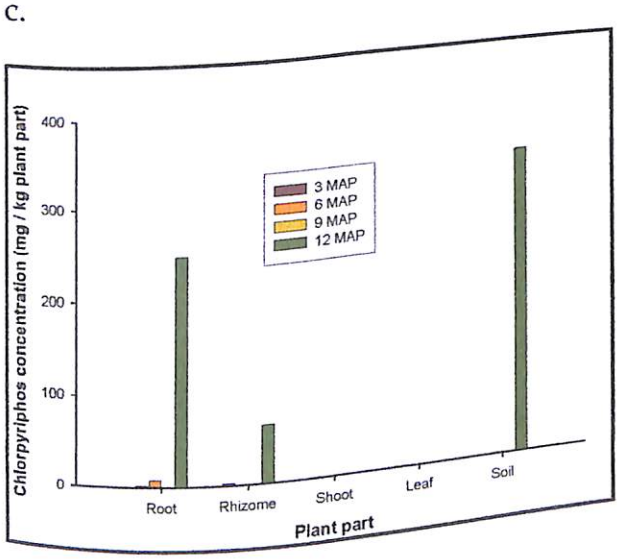
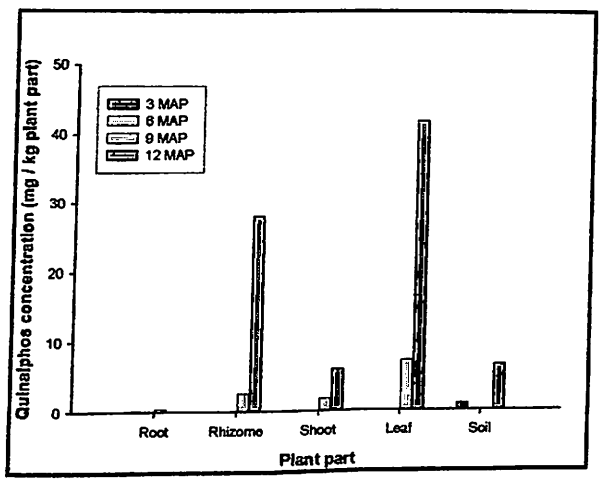
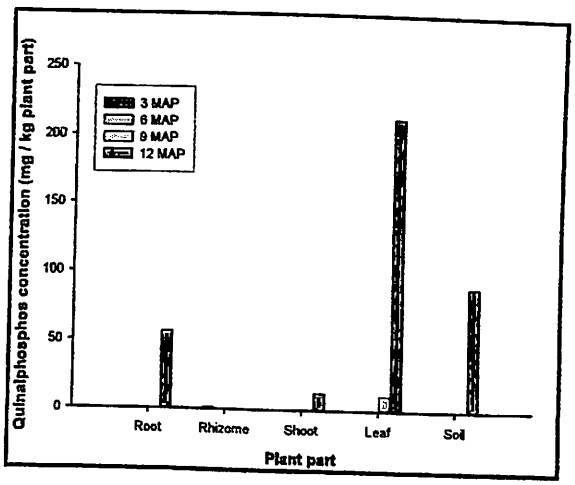


Figure - 4.26. Accumulation of quinalphos in plant parts and soil at (a). 0.5 x recommended dose; (b). recommended dose; (c). 2 x recommended dose

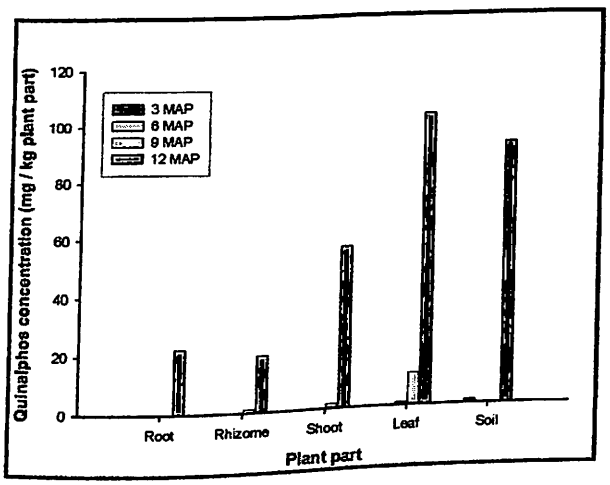
a.



b.



c.



Quinalphos also follows a similar trend as that of dimethoate (Figures 4.26 a- c). It was found that though these pesticides are systemic and applied on plants rather than soil, high levels and continuous application may lead to these getting accumulated in the soils. As quinalphos is weakly held by soils compared to the other pesticides in the study, this in due course will lead to pollution of water bodies in the region. The experiments show that under high doses there is a gradual

accumulation of all the pesticides in soils and plant bodies. In soils this will either be degraded or leached and in plants the decomposition is very slow as accumulated levels were seldom found to decrease in any of the experiments during the analyzed period. The plant accumulated pesticides have a high potential for bioaccumulation as it moves along the food chain. Hence it is always advisable to adopt methods of pest control with no or minimum levels of pesticides.

4. Conclusion

The soil reaction in CHR was found to vary between 3.56 - 6.98 and nearly 65 % of the samples were found to be very strongly to extremely acidic. Soil organic carbon in the soils were very high in most of the soils, but the restricted decomposition at low temperatures leads to wide C:N ratio in these soils. The soils were also adequate to high in P and K.

The adsorption studies reveal that soils of CHR have good pesticide retention capacity. A comparison of the linear plots of specific adsorption against the equilibrium concentration for the studied pesticides shows that the adsorption obeys more of a multilayer equilibrium (Freundlichs isotherm) than a monolayer one (Langmuir model). The adsorption isotherms also show that though the amount of maximum pesticide adsorbed decreases with pH, the binding strength increases. Dimethoate at higher pH, quinalphos at lower pH and chlorpyrifos at all pH are retained by strong chemical bonding. Thermodynamic assessments gave negative values of ΔG° indicating that the adsorption process of pesticides in soil is a spontaneous process.

Among the different pesticides used in the study, the soils showed maximum adsorption potential for chlorpyrifos followed by dimethoate ≈ quinalphos and the lowest kinetic rate constant (K_1) of adsorption was for quinalphos. The column experiments shows that under continuous leaching conditions dimethoate, chlorpyrifos and quinalphos are removed to the tune of 59.63%, 36.60% and 95.43% respectively. Chlorpyrifos with strong chemisorptions and high energy of binding was found to be the minimum leached pesticide in these soils. On continuous application of leachate, bases will be leached out leading to a decrease in soil pH. As the adsorption in the studied soils are essentially organic matter driven, decrease in soil reaction will alter the variable charges on these organic colloids and reduce the binding strength leading to increased desorption and leaching. These observations

point to the fact that immediate heavy rains / irrigation after pesticide application in these areas will leach most of the applied pesticides to ground water or water bodies thereby polluting them.

The field experiments show that under high doses there is a gradual accumulation of all the pesticides in soils and plant bodies. In soils this will either be degraded or leached and in plants the decomposition is very slow as accumulated levels were seldom found to decrease in any of the experiments during the analyzed period. The plant accumulated pesticides have a high potential for bioaccumulation as it moves along the food chain. Hence it is always advisable to adopt methods of pest control with no or minimum levels of pesticides.

Summary & Conclusion

The study was carried out in Cardamom Hill Reserves (CHR) of southern Western Ghats to assess the land degradation in terms of pesticide persistence, effect on physiochemical characteristics of the soil, toxicity to soil organisms, pesticide effect on forest trees and microflora. Sample collection was carried out by dividing the CHR area in to 198 grids, each of size 2.5 km^2. Survey data on pesticide usage pattern and soil samples were collected from every 9^{th} grid (four samples per grid and four replicate for each sample). A total of 24 grids were sampled during the study. Soils were collected from 4 spots in the grid, with each spot having four replicates.

Major crops and land use pattern in CHR were identified to be cardamom (78. 125 %), mixed crops (9.38 %), tea/coffee (1.04 %) and banana/cocoa (2 %). Mixed crops included pepper, cocoa, tapioca and banana as the major components. The interactions with large scale farmers, small farmers, labourers, pesticide shop owners, Govt officials -revenue, agriculture and health authorities etc., revealed the pattern of use of pesticides in cardamom plantations of the region as organophospharus (45.6%), neonicotinoids (12.66 %), pyrethroids (12.66 %), unknown/not declared (18.99 %), carbamates (5.06 %), GABA receptor blockers (2.53 %) and organochlorides (2.53 %).

The soils were subjected to pesticide residue analysis and 11.9 % of the soil samples contain some or other pesticides. Among the pesticides detected, 39.1% of samples showed the presence of chlorpyrifos, 26% showed quinalphos, 21.7 % samples showed the presence of both chlorpyrifos and quinalphos and 13 % samples showed the presence of arsenous acid.

Soil avoidance behaviour assays were carried out using soil invertebrates, in order to assess the suitability of soils for the survival of soil organisms. We have standardized the assay using *Eisenia fetida* as model organism using chlorpyrifos as a candidate chemical. Further soils from CHR were assessed using this assays. The assay establishes the avoidance of an unfavorable environment by soil invertebrates leading to aggressive movement of earthworms from the pesticide contaminated chamber.

Along with avoidance behaviour assays, basic toxicity studies were conducted using chlorpyrifos - the highly used organophosphate - on the earthworms *Eisenia fetida* as model organism. Toxic effects of chlorpyrifos on *Eisenia fetida* was

established with parameters such as mortality rate, weight loss, lipid peroxidation levels, total protein levels and glutathione. Histopathology of the exposed organisms were carried out to ascertain the toxicity effects. Significant decrease in weight, increased lipid peroxidation levels, decreased protein levels and decreased glutathione levels were observed in the exposed organisms. The histopathology of the exposed organisms showed protein degradation, cell membrane damage, cellular infiltration and inflammation. The microbial dehydrogenase assay was conducted to ascertain the soil health in terms of the microbial activity in soil. Control soils from normal unattended areas in the same agro-ecological conditions showed significant microbial dehydrogenase activity. Microbial activity in CHR soil samples were significantly lower compared to control soils.

Photosynthetic efficiency test and karyotype analysis were conducted to identify the stress level of common plant species in CHR under continuous pesticide application. Species used for the study were *Persea macrantha, Toona ciliata, Cullenia exarillata, Vernonia arborea* and *Artocarpus heterophyllus.* Chlorpyrifos, quinalphos and dimethoate were the pesticides used in this study. No direct effect of pesticides was observed on photosynthetic efficiency in the plants studied. Karyotype analysis of *Artocarpus heterophyllus* was noted with the presence of ball metaphase. Ball metaphase is a form of mitosis with characteristically clumped chromosomes. It indicated direct destructive effects of pesticides at the chromosome level

Soil samples from seventeen grid samples were subjected to microbial analysis. There were about 169 isolates belonging to 25 genera consisting of 55 species of fungi, 25 different types of actinomycetes and 40 bacterial isolates. Out of a total of 40 bacterial isolates, four were found to have resistance and degradation properties as indicated by the maximum clear zone formation around the colonies. Out of four bacterial colonies only three were identified up to the species level; *Bacillus subtilis, Micrococus varians* and *Bacillus licheniformis*

Out of 169 fungal isolates, 61 had resistance and degradation properties as indicated by clear zone formation and the other colonies are showing the pesticide resistant. A subset of 61 fungal isolates with high pesticide resistance and degradation properties were identified; *Aspergillus niger, Blastomyces dermites, Cladosporium herbarum, Colletotricum gloesporioides, Paecilomyces sp.,* and *Penicillium*

crysogenum. Further studies with these isolates may help to develop novel microbial consortia for pesticide degradation in soils.

The soil pH in CHR varied between 3.56 - 6.98 and nearly 65 % of the samples were very strongly to extremely acidic. Soil organic carbon in the soils were very high in most of the soils, but the restricted decomposition at low temperatures leads to wide C:N ratio in these soils. The soils were also adequate to high in P and K. The adsorption studies reveal that soils of CHR have good pesticide retention capacity. The adsorption isotherms also show that though the amount of maximum pesticide adsorbed decreases with pH, the binding strength increases. Dimethoate at higher pH, quinalphos at lower pH and chlorpyrifos at all pH are retained by strong chemical bonding. Thermodynamic assessments gave negative values of ΔG° indicating that the adsorption process of pesticides in soil is a spontaneous process.

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To conclude, though the soils have good pesticide retention capacity, degradation takes at least 1 month time with an initial slow phase. Being in the humid tropics

with high rainfall, there is a high risk potential for a major portion getting leached to ground water and slow decomposition of plant accumulated pesticides has a potential for bioaccumulation as it moves along the food chain.

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