WATER QUALITY ASSESSMENT OF THE MANASI ESTUARY, VENGURLA, MAHARASHTRA CENTRAL WEST COAST OF INDIA

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ABSTRACT

Water quality of Manasi estuary, a small brackish water biotope ($15^0$51'N 73^0 36'E) on the central west coast of India is one of the threatened estuary for domestic pollution. The discharge of domestic wastes by nearby local inhabitants is a major problem of the estuary. The water quality assessment in response to physicochemical parameters such as transparency, temperature, salinity, pH, dissolved oxygen etc as well as nutrients like nitrate, nitrite, phosphate showed significant spatial and temporal variations. The water temperature was highly influenced by atmospheric temperature. The transparency was found lowered due to presence of suspended matter at the polluted station near human community. Variation in the salinity were more conspicuous than the other analysed parameters. The land drainage and the sea-estuary interaction influences salinity distribution. Dissolved oxygen concentration was ow at human settlements. Nutrients (NO_3-N, NO_2-N, P-PO_4) showed significant spatial and temporal variations and higher values were observed at the polluted stations.

Keywords: Manasi estuary, Water assessment, polluted stations, Central west coast.

INTRODUCTION

Central west coast of Maharashtra shows a small chain of brackish water systems. The estuaries are smaller in size but important breeding station and nursery grounds for commercially important fishes. Manasi estuary a small brackish water biotope (15051'N 730 36'E) with luxuriant mangrove vegetation on the Vengurla coast and important from the fishery and seed resources point of view. This estuary serves as life line for the local community the estuary

Vengurla city is located on the bank of Manasiriver and estuarine area is mainly occupied by local fisherman community and considered as polluted area due to heavy discharge of

domestic waste. In the absence of adequate facilities for the sewage disposal and sullage from human community around the estuary is greatly discharged directly into brackish water.

This estuary has some religious importance due to the Manasishwartemple which is situate at the upstream region. The tidal influence is 2.5 km both the sides of estuary shows scattered mangrove vegetation with dwarf mangroves and also some rare species such as *Bruguieracylindrica* also present in this estuary.

Morphological changes near estuary mouth are also important as it influences the physico-chemical characteristics of the estuary. The establishment of snad bar resulting from immense coastal drift across the estuary mouth and its removal through specific intervals is common in the biotope since the total occlution of the mouth for longer duration results in the esturine overflow. The marine influence is visible when the mouth of estuary gets open.

There is very little information available on the water quality assessment of this estuary hence, present work was undertaken to evaluate seasonal water quality assessment of the estuarine as well as adjoining coastal region of Manasi estuary. The present work aims to get an insight in to the hydrography of this important estuary.

MATERIAL AND METHODS

Frequent monthly visits were carried out for surface and bottom water samples collection for one year between April 2017 to March 2018 from four different stations in the estuarine and coastal region of Manasi estuary. Station I was upstream of estuary, Station II at the middle, Station III at estuarine mouth and Station Iv on the sea shore. Water samples collected using plastic bucket from the surface and meyer type sampler used to collect samples from the bottom. Hydrological studies were carried out using standard methods (Grasshoff*et al*., 1983, APHA, 1985). ANNOVA test used for statistical analysis.

RESULTS AND DISCUSSION

Temperature

Temperature values reflects a great climatic variations. The maximum temperature was observed during the pre-monsoon period in air, surface and bottom water at all the studied stations (Table 1). . The decline in temperature was observed at the onset of monsoon where as it was reached to minimum during July-September. The air temperature fluctuated from 24^0C (Sep) to 33^0C (April). The maximum water temperature (32^0C) was recorded for both surface and bottom water during the month of April. The longitudinal distribution of mean atmospheric temperature as well as mean surface water temperature in the estuarine region was maximum at Station II and minimum at Station IV (Table 1). The influx of freshwater into the estuarine system is one of the factors in the lowering down the water temperature as well as cold water from the sea could be another significant factor. Similar kind of observation were reported by several workers in other estuarine system (Murugan and

Ayyakkannu, 1991in Uppanar Backwater, Rao and Sarma, 1995 in GosthaniEstuary and by ThillaiRajasekar*et al.*, 2005 in Coleroon Estuary).

Transparency

A significant difference ($P<0.01$) was observed between the transparency in the studied stations. Highest values were recorded twice in a year i.e. during February as well as in the month of November (Table 1). During the active monsoon season the intensity of solar radiation was minimum as the estuary flooded with siltborne surface runoff, the transparency was recorded at its lowest (Table 1). Turbidity seems to be a primary factor for the penetration of low light as noticed in other monsoon fed tidal estuaries (Chandran and Ramamoorthi, 1984; and Mishra *et al*., 1993).

pH:

The pH of the surface water ranges from 5.89 (November) at Station I to 8.73 (January) at station IV. The quantity of humic material in colloidal suspension, which is slightly acidic, is being transported through streams and rivers. These colloidal particles gets coagulated upon meeting sea water and the pH shifts towards the alkaline (AnilaKumary*et al*., 2007) .Hencethe values were greater towards downstream. Wide interstation variations occurred both in surface as well as bottom waters ($P<0.01$). Similar kind of reports were recorded earlier in the Indian estuaries (Chandran and Ramamoorthy, 1984; Upadhyay, 1988;

Murugan and Ayyakkannu, 1991). At the bottom the values ranged from 5.85 (station I) to 8.65 (Station IV). The higher value at bottom could be attributed to the vertical stratification of the water regards to salinity and DO.etc. there is no any seasonal pattern observed in the pH values.

Salinity

Salinity is the most conspicuous parameter than other hydrological parameters. The salinity showed more annual variations (Table 1). Surface salinity increased from $0.09X\ 10^{-3}$ at station I to $35.67X10^{-3}$ at station IV where as the bottom water salinity ranged from $0.15X10^{-3}$ station I and $34.98X10^{-3}$ at station IV.The fluactuation in the salinity was due to mixing of fresh water and sea water. Salinity was found maximum during the premonsson period at all the stations due to high evaporation, sea water dominance and cessation of freshwater flow from the upper reaches (Table 1).

Dissolved oxygen

The dissolved oxygen concentration in the surface layer ranges from 1.56 (station I) to 6.12 ml/l (Station III). The DO values of bottom was varied from 1.60 (station II) to 6.07 ml/l (station IV). The vertical gradient between surface and bottom was prominent at all the studied stations through out the year. The less oxygen content was observed at the bottom water and its concentration was more pronounced in fresh water dominated waters. (Table

1). The highest values at the surface could be due to the planktonic photosynthetic activity. The lower values of oxygen concentration at station II might be due to introduction of organic wastes and its decomposition.

Nutrients

The amount of nitrate nitrogen content in both surface and bottom water was greatly varied with respect to stations, months and seasons. The concentration ranges between 0.18 and 13.98 µmol/l at the surface water whereas 0.21 and 13.10 µmol/l at the bottom (Table 2). The lowest concentration was found at station IV in the marine zone while maximum amount was observed at station II.

The maximum amount of nitrate nitrogen could be due to possible input of high sewage disposal . Increased nitrate nitrogen values was also reported from Adayar mangrove are (Selvam*et al*., 1994) due to organic matter decomposition.

The nitrite content was found lower than nitrate however it also follows similar fluactuation trend. The variation was not significant at all stations. The mean concentration were high at Station II and lower at Stan I (Table 2). The role of freshwaterinput, rainfall and land drainage is of paramount importancein this context. The higher values at station II could be due to predominant sewage pollution. Another important factor is degradation of organic matters that might leads in the formation of appreciable amount of nitrite.

The higher concentration of phosphate phosphorus content in the surface and bottom (3.60 µmol/l and 2.71 µ mol/l) respectively recorded at station II. The lowest amount in both surface and bottom water was found at marine stations. The higher concentration of phosphate phororus content was recorded during the monsoon period (Table 2).

The rise in phosphate phorphorus during monsoon could be due to the rainfall and discharge of sediments from river water. High density of phytoplankton and the resultant increased utilization of phosphate might have resulted in the decrease of the nutrient during the pre-monsoon and late post-monsoon months (Padmavathi and Satyanarayana, 1999; Renjith*et al*., 2004).

Conclusion

The present study revealed the harmful effects of waste disposal on the water quality of the estuary. The marked increase in nutrients and a significant decrease in the dissolved oxygen at station II compared to all the other stations. The effect of this pollution are greatly reduced at station III.

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Table 1. Range and mean of various physico-chemical parameters at four stations

Parameters		Station I			Station II			Station III			Station IV		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Transparency cm		12.1	70	33.25	9.17	80.73	42.07	15	106	54	--	--	--
Water Temp ^{0}C	S	28	30.10	29.33	28.10	31.9	29.54	27.2	32.3	29.38	23.10	31.50	28.32
	B	28	30.08	29.21	27.50	31.7	29.20	26.89	30.70	29.18	23.91	30.90	28.36
pH	S	5.89	7.48	6.69	6.42	7.54	7.02	6.73	8.15	7.28	7.53	8.73	8.19
	B	5.85	7.65	6.89	6.42	7.80	7.42	7.24	8.23	7.74	7.50	8.65	8.17
Salinity S 10^{-3}	S	0.09	1.52	0.70	0.64	3.01	1.63	0.65	8.38	3.12	24.14	35.67	31.11
	B	0.15	7.20	1.49	0.94	25.10	8.50	1.28	32	13.55	24.12	34.98	31.20
Dissolved Oxygen ml/l	S	1.56	6.02	4.97	1.64	5.77	4.26	2.20	6.12	4.57	3.28	6.05	5.14
	B	2.21	5.70	4.67	1.60	5.23	3.89	2.20	5.40	4.07	3.30	6.07	4.99

S:- Surface B:- Bottom

Table 2. Range and mean of various nutrients at four stations

Parameters		Station I			Station II			Station III			Station IV		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Nitrate nitrogen (µmol/l)	S	1.14	4.25	2.18	0.86	13.98	5.93	0.56	7.0	2.38	0.18	1.20	0.71
	B	1.01	4.01	1.93	0.85	13.10	4.64	0.32	4.51	1.51	0.21	1.20	0.70
Nitrite nitrogen (µmol/l)	S	0.32	2.48	1.36	0.16	5.64	2.53	0.34	2.98	1.40	0.17	0.67	0.49
	B	0.33	2.50	1.24	0.33	4.32	4.10	0.33	2.66	1.03	0.17	0.75	0.46
Phosphate phosphorus (µmol/l)	S	0.15	1.81	0.74	0.76	3.60	1.62	0.45	2.12	1.10	0.21	0.91	0.53
	B	0.15	1.82	0.68	0.45	2.71	1.17	0.23	1.51	0.69	0.21	0.91	0.53

S:- Surface B:- Bottom

INSECT HERBOVIRE COMMUNITY OF AMBA RESERVED FOREST KOLHAPUR DISTRICT, (MS), INDIA

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ABSTRACT

Amba reserve forest is welkown for its species richness and high rate of endemism. It is one of the popular tourism point of the Western Ghats and falls between 15^0 43' to 17^0 10' north and longitude 73^0 40' to 74^0 42' east and 691.3 meters above Mean Sea Level. The insect herbivore community of this forest is explored over the period of 2 years. The sampling was done at each station in morning (07.00am to 11.00 am) and evening (05.00pm to 8.00pm). Investigation on the insect herbivores reported 42 species of insects belonging to the orders Isoptera, Hemiptera, Thysanoptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera from 24 families. Insects are known for their wide host range and also for absolute specificity with single plant species. Insects display different types of foliage feeding, according to their taxonomic groups.

Key words:- Amba reserve forest, Insect herbivores, foliage feeding,

INTRODUCTION

Forests plays significant role in maintaining life on earth and it is precise renewable natural resource and contributing substantially in the wealth of nation. Forest vegetation is composed of plant communities or units of vegetation developed and arranged in accordance with definite biological laws and it is not an aggregation of trees and other plants brought together by chance (Toumey and Korstian, 1947).

Forests exhibit numerous examples of floral and faunal diversity, especially tropical forests as they are well known for their high species richness and high rate of endemism **(**Mittermeier *et al*., 1998, 2003; Kier *et al*., 2005; Brooks *et al*., 2006). Tropics include many continents i.e. some portion of Asia, Australia, Africa, North America and South America and several Islands in the pacific, Atlantic and Indian oceans. These tropical countries are grouped under three major regions Asia-pacific, Africa and Latin America. These countries cover a considerable region of earth's land surface nearly 37%, comprising about 4800 million ha. (Nair, 2007). The overall structural, floral and faunal composition of tropical

forests varies according to the topographical conditions. For instance, low land evergreen forests, upper & lower montane forests and mangrove forests differ to a great extent in their in general structure and floristic composition, are often called as forest formations.

Indian forests are varied in composition and character. They are spread over the entire country from Kashmir Himalayas in the north to Cape Cameron in South, Saurashtra in the West to Assam in the east of India. They exhibit a great diversity of forms, which is due to variety of factors of climate, physiography, geology, soil, water conditions and biota. In the world categorization of Indian forests are noted as monsoon & dry forests and thorn bush and Savanna (Puri, 1960). Indian forests have over 30,000 species of higher plants and 600 species of Pteridophytes. Of the overall figure of higher plants, Dicotyledons alone represented by 11, 124 species. In India Monocotyledons occupy a fairly prominent place, behind every seven Dicotyledonous there is one species of monocot. There are more than 100 species of Bamboos and 25 species of Conifers in India. More or less the Indian vegetation is considered as monsoon tropical (Puri, 1960).

The earlier studies on the phytophagus insect fauna of tropics showed that the tropical forest is rich in species diversity (Barbosa & Schultz, 1987; Nair, 2007). But insects associated with natural stands and artificial stands displays different levels of diversity, population fluctuations, % of folivory and diet breadths according to the availability of host plants and favorable climatic conditions. An overview of tropical forest insects reveals that all insect orders are present in the tropical forest ecosystem except Grylloblatoidea and Mantophasmatodea. However, some insect orders are dominant, that is more abundant and economically important because of their negative impact on trees. The prevalent orders are Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Isoptera and Orthoptera (Nair, 2007).

The diversity of woodland insects is also reflected in their feeding habits. Generally every organic material in the natural stands is eaten by one or other insect species. Consumption of a plant material is a subject of great economic importance as well as biological importance (Price *et al*., 1991; Hunter *et al*., 1992; Bernays and Chapman, 1994). Insects cause damage to plant in every stage of the growth from the seed to the finished products. It ranges from 0% to 100% consumption of plant material. Regupathy *et al*., (1995) has given the common categories of insect damage and their signs associated with forest and shade trees

At present day's situation, except, herbivores of agricultural crops and their damage, no in formation is available on the phytophagus insects of plantations and forests of Kolhapur district. The present study explores the insect herbivore community of Amba reserve forests. To our knowledge, this is one of the first extensive studies of insect herbivores from this part of Northern Western ghats.

STUDY AREA

Observations were recorded at Amba reserve forest in Kolhapur district It has been divided into 12 talukas and 4 sections for managerial reason. For the present study, Amba reserve forest representative forests from Western Ghats that comes in Kolhapur district of Maharashtra have been selected.

Amba Reserved Forest most popularly considered as tourism point by the people of Kolhapur district. It comes in Taluka Shahuwadi of district Kolhapur. It is situated at North-

West of Kolhapur District. It consist tropical semi-evergreen forest of north Western Ghats. The geographical area is 318.16 ha. The average annual rainfall noted was 6000 mm. Temperature of this region during summer, winter and Rainy Season ranges from 25 ^{0}C to 38 ^{0}C, 10 ^{0}C -30 ^{0}C and 15 ^{0}C -30 ^{0}C respectively. The soil type is lateritic, red and yellowish to red-brown soil observed in the area. The area under study has part of mixed semi evergreen and moist mixed deciduous forest exhibits tremendous plant diversity and good vegetative cover and harbors vegetation types ranging from seasonal grasses, herbs and climbers to perennial herbs, shrubs and trees. Rainy season enjoys most greenery in the study region. The sampling of the specimens was made at different localities by considering all type of habitats.

METHODOLOGY

Extensive surveys were carried to find out insect herbivore complex during present study. The sampling was done at each station in morning (07.00am to 11.00 am) and evening (05.00pm to 8.00pm). During the sampling, insect habitats on and within the trees and their associated insect groups were observed and sampled accordingly. Major habitats of insect herbivores and evidences of their activity on different parts of trees were identified using standard literature (Leather, 2005).

Sampling stations and field survey

All the sampling stations from Amba reserve forest were selected for study exhibits varied physiographic units.

Sampling Duration and strategies

The field surveys were conducted during the years 2015 to 2017. The sampling was carried out during all the seasons. However, rainy season, the period from June to September found to be most suitable for collections, observations and to understand the association of insect herbivores within study sites. Because several insects start their activity or life cycle with the first downpour of monsoon.

Killing, preservation, mounting and labeling

The preservation, mounting and labeling of insect was followed as per Alfred and Ramkrishna (2004).

Identification

The identification of insect specimens was made with the help of available literature

The specimens not identified up to generic level were arranged up to their respective families by using the relevant Fauna of British India.

Result and Discussion:

In the context of insect herbivores, their host range and damage to economically important forest plants, a critical assessment was made with reference to the menace of insect pests in Amba reserve forestarea of Kolhapur district. This study is an attempt to assess the present insect pests of indigenous material in the virgin forests.

Investigation on the insect herbivores yielded 41 species of insects belonging to the orders Isoptera, Hemiptera, Thysanoptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera.from 24 families Not only insect show high diversity among all animals, it also reflected in their

habitat and feeding habits. Associations of insect pests with specific plant organs were also recorded. They were found on flowers, fruits, foliage, stem and roots. Among these folivorous, insects dominated the remaining groups.

The insect herbivores of selected economically important forest plants from Amba reserved forest of Kolhapur district was documented (Table 1,).

Generally sap feeders belong to the order Hemiptera. Some examples were also reported from the order Diptera and Thysanoptera. Shelter feeding type of feeding includes web enclosed feeding, leaf tying, leaf rolling, crinkled leaves, leaf and petiole galls. Several lepidopterous caterpillars web foliage and feed on the enclosed foliage. Early stages of many lepidpterous insects feed on the surface of the leaf. Caterpillars of *Thiacidas postica* feed on the surface of *Z. jujuba* leaves. Insects feed on the green content between the veins, leaving veins as a skeleton. Developing stages of *Eutectona machaeralis* and many lepidopterous insects feed on the soft material between the networks of veins, which results into skeletonization of leaves. Insects feed greedily on the foliage leaving larger veins behind. Several lepidopterous caterpillars feed in this way.

Roughly circular mined areas were observed on the foliage of *Anacardium ocidentale, Terminalia arjuna, Terminalia tomentosa, Syzygium cumini, Tamarindus indica* and *Mangifera indica.* For instance Caterpillars of *Acrocercops phaeosphora* forms a blisher like mine at the central portion on the leaves of *Syzygium cumini.* Leaf feeders include large proportion of forest insects distributed within the orders Coleoptera, Lepidoptera, Hymenoptera, Orthoptera and Diptera. Insects display different types of foliage feeding, according to their taxonomic groups.

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Table 1.Insect herbivores of forest plants from Amba reserve forest of Kolhapur district

Sr. No	Scientific Name	Order	Family	Common Name	Principal trees attacked	Affected plant parts
1	*Odontotermes obesus* Rambur ?	Isoptera	Termitidae	White Ant	*Careya arborea, Mangifera indica, Terminalia tomentosa, Terminalia arjuna, Nothapodytes nimmoniana, Bridelia retusa*	Stem
2	*Trioza fletcheri minor* Crawford	Hemiptera	Psyllidae	Psyllid	*Terminalia arjuna, Terminalia tomentosa*	Leaf
3	*Trioza jambolanae* Crawford	Hemiptera	Psyllidae	Psyllid	*Syzygium cumini*	Leaf
4	*Pauropsylla depressa* Crawford	Hemiptera	Psyllidae	Psyllid	*Ficus racemose*	Leaf
5	*Toxoptera oedinae?*	Hemiptera	Ahididae	Aphid	*Mangifera indica*	Leaf
6	*Rhipiphorothrips cruentatus* Hood	Thysanoptera	Thripidae	Thrips	*Careya arborea*	Leaf
7	*Austrothrips cochinchinensis* Karny	Thysanoptera	Phlaeothripidae	Thrips	*Calycopteris floribunda*	Axial Buds
8	*Liothrips karyni* Bagnall	Thysanoptera	Phlaeothripdae	Thrips	*Piper nigrum*	Leaf
9	*Eutectona machaeralis* Walker	Lepidoptera	Pyralidae	Teak Skeletonizer	*Tectona grandis*	Leaf
10	*Orgyia postica* Walker	Lepidoptera	Lymantridae	Tussock Moth	*Tectona grandis*	Leaf
11	*Orgyia australis* Walker	Lepidoptera	Lymantridae	Tussock Moth	*Syzygium cumini, Terminalia bellirica*	Leaf
12	*Acherontia styx* Westwood	Lepidoptera	Sphingidae	Deaths Head Hawk Moth	*Tectona grandis*	Leaf
13	*Psilogramma menephron* Cramer	Lepidoptera	Sphingidae	Hawk Moth	*Tectona grandis*	Leaf
14	*Parasa lepida* Cramer	Lepidoptera	Limacodidae	Blue Striped Nettle Grub	*Careya arborea, Butea monosperma, Bridelia retusa, Sapium insegne,*	Leaf

					Acacia auriculiformis, Terminalia arjuna, Terminalia tomentosa, Mangifera indica, Syzygium cumini.	
15	*Metanastria hyrtaca* Cramer	Lepidoptera	Lasiocampidae	Diamond back Caterpillar	*Bridelia retusa, Terminalia tomentosa, Syzygium cumini, Terminalia arjuna*	Leaf
16	*Trabala vishnu* Lefebure	Lepidoptera	Lasiocampidae	Lappet moth	*Terminalia arjuna, Terminalia tomentosa, Syzygium cumini, Butea monosperma,Eucalyptus globulus, Lagerstroemia sp.*	Leaf
17	*Spodoptera litura* Fab.	Lepidoptera	Noctuidae	Cutworm	*Tectona grandis, Mangifera indica*	Leaf
18	*Carea angulata* Fab.	Lepiodoptera	Noctuidae	Nolid moth	*Syzygium cumini*	Leaf
19	*Thiacidas postica* Walker	Lepidoptera	Noctuidae	Ber white Hairy Caterpillar	*Zizyphus jujuba*	Leaf
20	*Antheraea mylitta* Drury	Lepidopterqa	Saturniidae	Tasar Silk Moth	*Syzygium cumini, Terminalia tomentosa, Terminalia arjuna*	Leaf
21	*Actias selene* Hubner	Lepidopterqa	Saturniidae	Moon Moth	*Terminalia arjuna, T. tomentosa, Ficus bengalensis.*	Leaf
22	*Attacus atlas* Linn	Lepidopterqa	Saturniidae	Atlas Moth	*Sapium insegne, Holorrhena antdidysentrica, Embelia ribes*	Leaf
23	*Euthalia aconthea suddhodana* Frushthorper	Lepidoptera	Nymphalidae	Common Baron	*Mangifera indica*	Leaf
24	*Catopsilila pomona* Fab.	Lepidoptera	Pieridae	Common Emigrant	*Cassia fistula*	Leaf
25	*Catopsilia pyranthe* L.	Lepidoiptera	Pieridae	Mottled Emigrant	*Cassia fistula*	Leaf
26	*Jamides celeno* Walker	Lepidoptera	Lycaenidae	Common Cerulean	*Butea monosperma*	Leaf
27	*Acrocercops phaeospora* Meyrick	Lepidoptera	Gracillaridae	Leaf miner	*Syzygium cumini*	Leaf
28	*Acrocercops syngramma* Meyrick	Lepidoptera	Gracillaridae	Leaf miner	*Anacardium occidentale*	Leaf

29	*Dysphania percota* Swinhoe	Lepidoptera	Geometridae	Blue Tiger Moth	*Carallia brachiata*	Leaf
31	*Sinoxylon anale* Lesne ?	Coleoptera	Bostrichidae	False Powder Post Beetle	*Careya arborea*	Stem
32	*Psiloptera orientalis* Fab.	Coleoptera	Buprestidae	-	*Syzygium cumini*, *Acacia* sp	Bark
33	*Aeolesthes holosericea* Fab	Coleoptera	Cerambycidae	Stem Borer	*Terminalia tometosa, Terminalia arjuna, Albizia lebbeck, Albizia procera, Bridelia retusa*	Stem
34.	*Holotrichia fissa* Brenske	Coleoptera	Scarabaeidae	Chafer beetle	*Emblica officinalis, Bridelia retusa, Careya arborea, Zizyphus jujuba, Butea monosperma, Grewia sp., Terminalia tometosa, Terminalia arjuna, Syzygium cumini*	Leaf
35	*Holotrichia karschi* Brenske	Coleoptera	Scarabaeidae	Chafer beetle	*Syzygium cumini, Bridelia retusa, Acacia auriculiformis, Terminalia T. arjuna, T. Tomentosa*	Leaf
36	*Platypriya andrewesi* Weise	Coleoptera	Chrysomelidae	Hispa	*Zizyphus jujuba*	Leaf
37	*Monolepta signata* Olivier	Coeloptera	Chrysomelidae		*Zizyphus jujuba*	Leaf
38	*Myllocerus undecimpustulatus* Faust	Coeloptera	Curculionidae	Weevil	*Tectona grandis, Terminalia tomentosa, Terminalia arjuna Careya arborea*	
39	*Apoderus tranquebaricus* Fab.	Coleoptera	Curculionidae	Giraffe Weevil	*Terminalia tomentosa, Terminalia arjuna, Mangifera indica, Dimocarpus longan, Mammea suriga, Lagerstroemia, Aporosa lindleyana, Syzygium cumini, Anacardium occidentale, Grewia sp.*	Leaf
40	*Cyrtotrachylus dux* Boheman	Coleoptera	Curculionidae	Bamboo Weevil	*Dendrocalamus hamiltonii* and other species of Bamboo.	Young Sprouting Culm

41	*Leptocybe invasa* Fisher & La Salle	Hymenoptera	Eulophidae	Blue Gum Chalcid	*Eucalyptus globulus, Eucalyptus citriodora*	Leaf
42	*Xylocopoa aestuans* Linn.	Hymenoptera	Anthrophoridae	Carpenter Bee	*Ficus racemosa*	Branch

ALLELOPATHIC EFFECT OF RANMODI (CHROMOLEANA ODORATA L.) LEAF EXTRACTS ON ENZYMATIC ANTIOXIDANT MACHINERY OF SOME COASTAL PLANTS

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Abstract
The present study intended to investigate the effect of aqueous extract from Chromoleana odorata L. on the enzymatic antioxidant machinery of some mangrove and mangrove associate plants. The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), Peroxidase (POX) and polyphenol oxidase (PPO) were markedly affected due to leaf extract treatments of C. odorata. The activity of enzyme catalase exhibited almost three fold stimulation in Mangrove plant Sonneratia alba and Acanthus ilicifolius followed by a sand dune species Ipomoea pes-caprae. The activities of enzyme peroxidase and superoxide dismutase were stimulated in all the coastal plant species studied except Derris trifoliate. The allelopathic effects of C. odorata on the enzymatic antioxidants in the studied plants were might be due to the allelochemicals present in the leaf of C. odorata.
***Keywords**: Chromoleana odorata, superoxide dismutase, catalase, Peroxidase, polyphenol oxidase*

Introduction
Allelopathy is captivating and mystifying subject deals with plants interaction influenced by the chemical substances that they release into the environment (Machado, 2007). The allelochemical from the plants are released into the soil either through exudation from roots, leaching from aerial parts such as stem leaves or through decomposition of plant material (Inderjit et al., 2006). Allelopathic effect is multiple effect which affects cell division, plant hormone production, membrane permeability, pollen grian germination, pigment synthesis, uptake of minerals, photosynthesis, respiration, protein synthesis, nitrogen fixation and enzyme activities (Djurdjevic et al., 2012 & Mansour, 2013).

Allelopathy plays an important role in agricultural ecosystems leading to wide range of interaction between crop-crop, crop-weed and crop-tree (Zahida et al., 2006). sometimes these kind of interaction harmful to recipient plants but may provide some benefits to donor also (Adrain et al., 2000). In nature most of the plants secretes phytotoxic allelochemicals with strong allelopathic properties (El-Darier and Tammam, 2009). In the recent years allelopathic potential of many plants and weed have been investigated against different crops (Gulzar and Siddiqui, 2014). these plants releases waters soluble chemicals in the soil which inhibits the germination and growth of surrounding plants (Batish et al., 2007). These allelochemicals can also be used as natural herbicides as a biological control agents (Razzaq et al., 2012). The allelochemicals creates some sort of oxidative stress to the recipient plants. to defend these stress conditions, plants are equipped with several ROS scavenging enzymes such as superoxide

dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), polyphenol oxidase (PPO) which are activated to strengthen the plants against biotic and abiotic stresses.
Chromolaena odorata is rapidly emerging perennial ever green shrub inhabitant to south America and focal America. It acts as more destructive insidious weed in tropical Asian, African and in Australian countries. This genus is branded as Siam weed belonging to family Asteraceae and have ample assortment of allelopathic potential. *Chromolaena odorata* grows in many soil types. It forms opaque position as weed and prevent organization of other species both due to antagonism and allelopathic consequence. In the coastal sand dunes of Goa occurrence of the *Chromolaena odorata* has been noticed by Janarthanam and Dessai (2005). However, the allelopathic influence of this plant on various costal species has a not been so far investigated. So to know the possible bang of Siam weed on the coastal vegetation, the research problem was selected.

Materials and Methods

1. Procurement of leaf litter and seedlings.

Senescent leaves of *Chromolaena odorata* L. were collected from the coastal area of Sawantwadi taluka, Maharashtra.The leaf litter was preserved in polythene bags and stored in dry conditions.The fresh matured, insect and disease free leaves of *Chromolaena odorata*were collected from same place at the time of experimental work.

Seedlings of *Sonneratia alba*Sm**.**, *Acanthus ilicifolius*L., *Derris trifoliata* Lour., *Salvadora persica*L., *Crotalaria verrucosa* L., *Crotalaria retusa* L.*and Ipomoea pes-caprae*(L.) R. Br. were collected from Aronda, Sawantwadi taluka and potted in pots.

2. Preaparation of leaf leachate and leaf extract.

The senecent leaf litter was weighted, washed altogether with tap water and blotted to drythen 200g leaf material was soaked in 1 litre of distilled water for 24 hours at room temperature. Following 24 hours leachate was separated through Whatman No.1 filter paper. The leaf leachate (filtrate) was put away in icebox until utilized for additionally examines.

The fresh leaves were washed thoroughly with tap water, chopped into 1 cm long pieces and were grated with mechanical grater. The 200g ground leaf matter was weighted and soaked in 1 litre of distilled water for 24 hours and filtered through muslin cloth followed by then Whatman filter paper No 1. The aqueous extract was stored in a refrigerator until used for further studies.

Leaf leachate and leaf extract treatments have given to seedling pots by immersing pots into tray containing leaf leachate and leaf extract. When the surface soil of pot is wet then pots removed from the tray and kept in polyhouse. These treatments were continued for three weeks. Then the leaves of each plant species are harvested, dried, powdered and used for further analysis.

3 Enzymatic antioxidants

Catalase action was examined by following the technique for Luck (1974) as portrayed by Sadasivam and Manikam (1992). The dissolvable proteins in the chemical concentrate were resolved by the technique for Lowry *et al.* (1951) which has been portrayed before. The compound action is communicated as ΔOD min^{-1} mg^{-1} protein.

Peroxidase action was contemplated by the technique for Horiguchi, (1988). The solvent proteins in the compound concentrate were resolved by the technique for Lowry *et al.* (1951) which has been depicted before. The enzyme activity was expressed as Δ O.D. h^{-1} mg^{-1} protein.

Superoxide dismutase practice used to be resolved after the strategy portrayed by Giannopolitis and Ries (1977). The enzyme activity is expressed as ΔO.D. $h^{-1}mg^{-1}$ of protein.

The movement of an oxidative chemical, polyphenol oxidase, was examined spectrophotometrically by utilizing the extraction and test method proposed by Mahadevan and Shridhar (1982). The change in optical density was recorded for 1 min. The activity of enzyme is expressed as Δ O.D. $min^{-1}mg^{-1}$ protein.

Results and Conclusion

The effect of leaf extracts and leaf leachate extracts of *Chromolaena odorata* on the activity of enzyme catalase in some coastal plants is depicted in the Fig. 1. The activity is expressed on Inhibition/stimulation (%) over control. It is clear from the figure that the activity of enzyme catalase is stimulated in all the coastal plant species studies by the application of leaf extract and leaf leachate extracts of *Chromolaena odorata* and the stimulation is more pronounced in leaf leachate extracts. It is also noticed that the enzyme activity is exhibits almost threefold stimulation in Mangrove plant *Sonneratia alba* and *Acanthus ilicifolius* followed by a sand dune species *Ipomoea pes-caprae*.

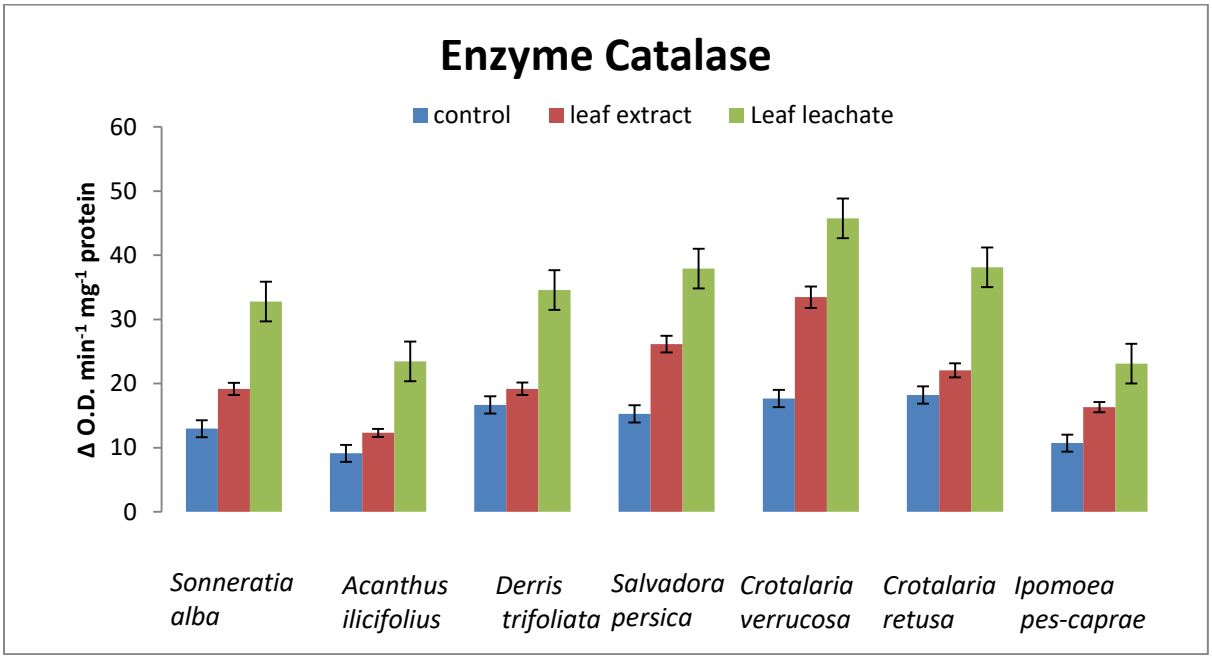

Fig.1. Allelopathic effect of *Chromolaena odorata*on catalase activity of some coastal plant

An improvement in Catalase action has been accounted for in one-of-a-kind examinations on allelochemicals technique of interest, that is, ferulic acid prolonged Catalase movement in maize seedlings (Devi and Prasad, 1996) and benzoic corrosive (acid) in cucumber cotyledons (Maffi *et al.*, 1999). In spite of the truth that evidence about allelochemicals actuated oxidative stress collectively with extended movement of antioxidant catalysts is growing (Rafael *et al*., 2005; Ye *et al*., 2006) however,in any case, little records is on hand approximately the systems via which allelochemicals instigate ROS association. Singh *et al*., (2016) also recorded

improvement catalase activity in sunflower plants subjected to *Nicotiana plumbaginifolia* leaf leachate extract.

Enzyme Peroxidase

The changes in the activity of enzyme peroxidase in some coastal plants subjected to leaf extract and leaf leachate extracts of *Chromolaena odorata* is shown in the Fig. 2. The activity is expressed on Inhibition/stimulation (%) over control. It is clear from the figure that the activity of enzyme peroxidase is stimulated in all the coastal plant species studies except *Derris trifoliata*, due to the application of leaf extract and leaf leachate extracts of *Chromolaena odorata* and the stimulation is more pronounced in leaf leachate extracts. It is also noticed that the enzyme activity exhibits almost two fold stimulation due to leaf leachate extract as compared to control.

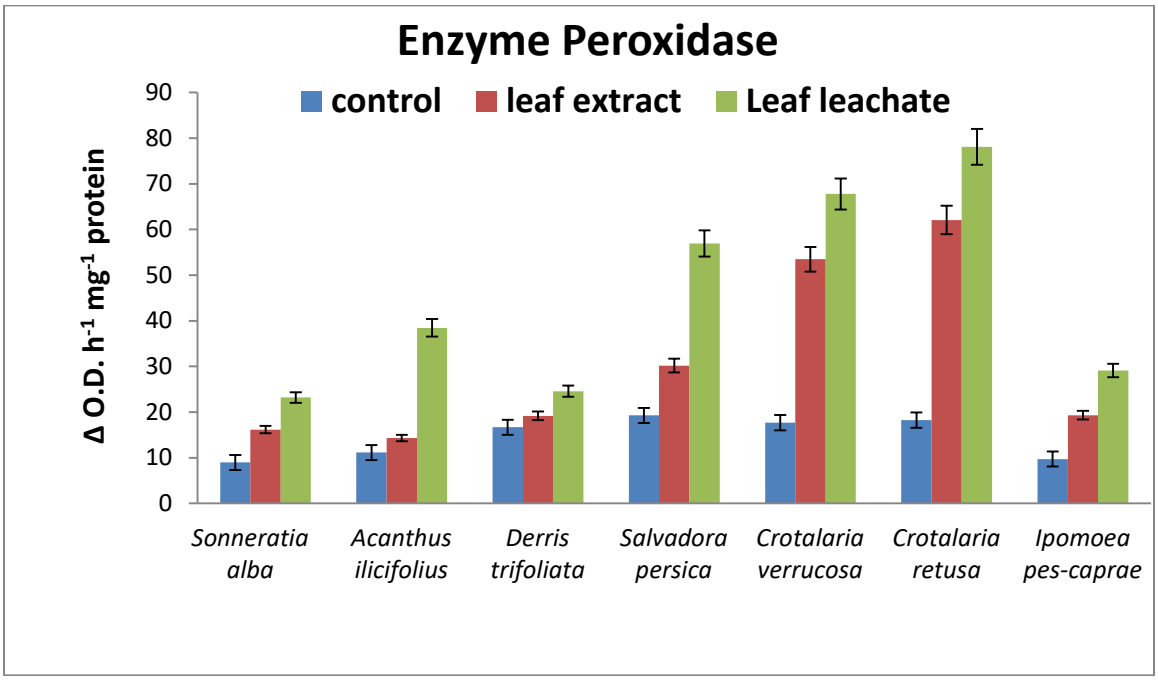

Fig.2. Allelopathic effect of *Chromolaena odorata*on peroxidase activity of some coastal plant.

According to Sairam and Srivastava (2002), hydrogen peroxide, a more stable ROS, can allocate diagonally organic membranes. The incitement of peroxidase movement in plants happens in light of numerous living and nonliving natural stresses (Casal *et al*., 1994). Under pressure condition various antioxidative chemicals expanded enormously to dodge the oxidative harm caused by ROS (Foyer and Noctor, 2003; Singh *et al*., 2009). Doblinski *et al*. (2003) and Cruz Ortega *et al*. (2002) also announced increment in Peroxidase activity. The increase in the activity of enzyme peroxidase as an antioxidative defense mechanism certainly proves the phytotoxic nature of *C. odorata* leaf extract and leaf leachate extract.

a. Enzyme Superoxide dismutase

The regulation in the action of compound Superoxide dismutase in some coastal plants presented to leaf concentrate and leaf leachate concentrates of *Chromolaena odorata* is appeared

in the Fig. 3. The activities corresponded on Inhibition on Inhibition/stimulation (%) over control. It is obvious from the assume that the movement of catalyst superoxide dismutase is fortified in all the seaside plant species studies about with the exclusion of *Derris trifoliata*, utilization of leaf extract and leaf leachate concentrates of *Chromolaena odorata* and the incitement is more articulated in leaf leachate extracts. It is also likewise seen that the compound movement of enzyme is exhibits almost two fold encouragement in leaf leachate extract when contrasted to control.

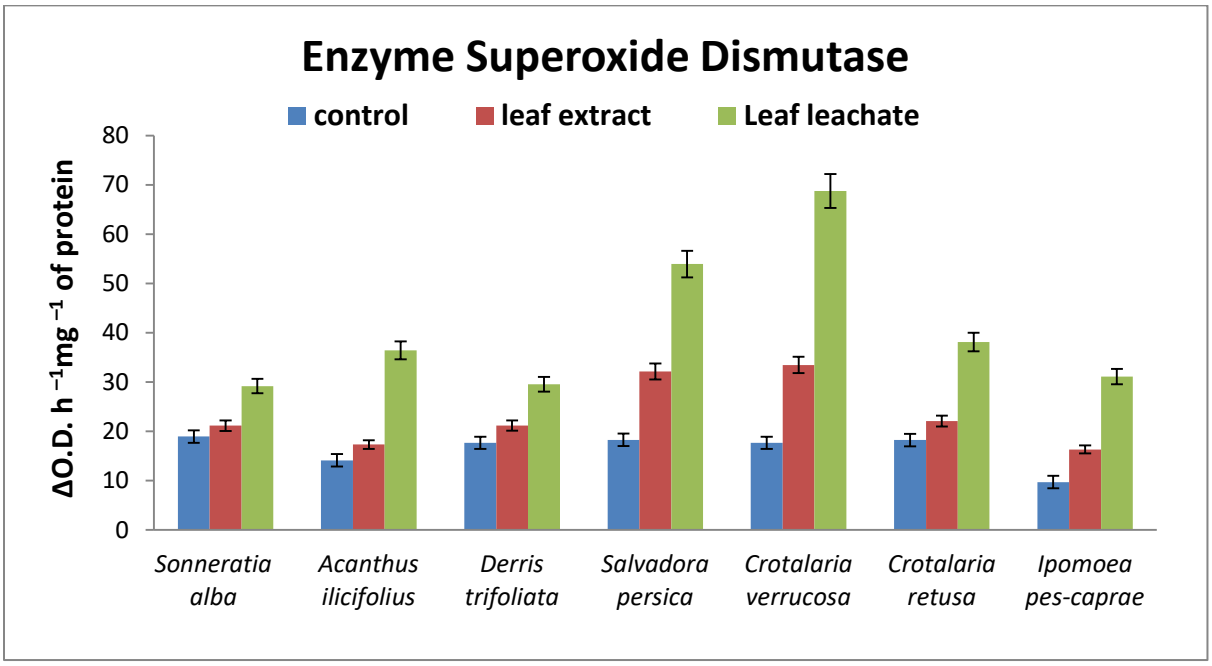

Fig. 3.Allelopathic effect of *Chromolaena odorata* on the activity of enzyme superoxide dismutase some coastal plant

Incensement in Superoxide dismutase bustle in present investigation is identical as evidence by Gomez *et al.* (2004) where they reported an enhancement in all superoxide dismutase enzymes of pea chloroplast by salinity treatments. According to Koca *et al*. (2007) saline stress leads to decline in superoxide dismutase enzyme activity in salt sensitive plants of *Sesame indicum* L. than salt tolerant ones (Akbar *et al*.,2009).

Enzyme Polyphenol Oxidase

The changes in the activity of enzyme Polyphenol oxidase in some coastal plants exposed to leaf extract and leaf leachate extracts of *C. odorata* is shown in the Fig. 10. The activity is expressed on Inhibition/stimulation (%) over control. It is clear from the figure that the activity of enzyme Polyphenol oxidase is stimulated in all the coastal plant species studies, by the application of leaf extract and leaf leachate extracts of *Chromolaena odorata* and the stimulation is more pronounced in leaf leachate extracts. It is also noticed that the enzyme activity is exhibits almost two fold stimulation in leaf leachate extract in contrast with control.

Recently, Li and Steffens (2002) have obtained direct evidence of such a responsibility for Polyphenol oxidase in plants. The mode of deed projected for Polyphenol oxidase is based on its capacity to oxidize phenolic derivaties when the tissue is smashed (Melo *et al.*, 2006).

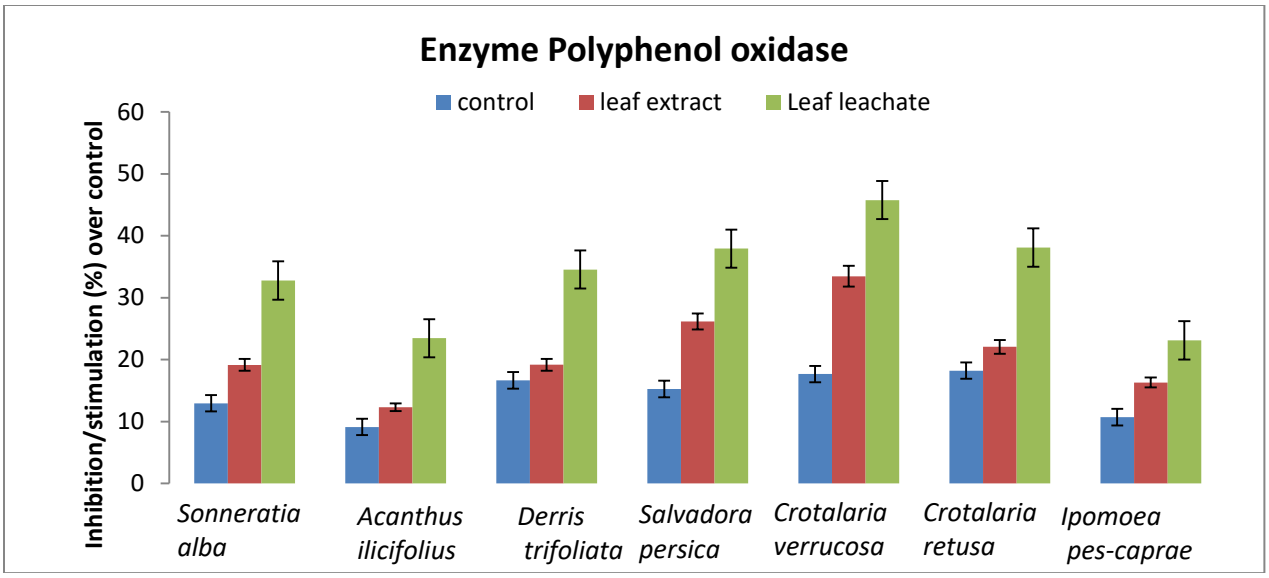

Fig. 4.Allelopathic effect of *Chromolaena odorata*on the activity of enzyme Polyphenol oxidase some coastal plant.

Increasing in protective enzyme activities ensured that the plants treated can effectively protect the membrane from the active oxygen and led to the stability of the membrane, so that the seedlings were able to acclimate to the allelopathic stress by adjusting the activities of SOD, CAT and PPO as Suggested by Yang *et al*., (2009).

Conclusion

The appliance of leaf concentrate and leaf leachate of Siam weed may contributes in the increase in H_2O_2 that may be further attributed to the resulting reactive oxygen species (ROS) by allelochemical stress. The elevation in the enzymatic antioxidant can certainly proves the strong antioxidant defense mechanism of coastal species studied and the phytotoxic nature of *C. odorata* leaf extract and leaf leachate extract.

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INSECT HERBIVORES, THEIR DISTRIBUTION AND HOST RANGE IN THE RADHANAGARI AND PLANTATIONS FOREST OF, KOLHAPURDISTRICT, MAHARSHTRA

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ABSTRACT

Radhanagari forest is situated at latitude range of 16^0 15' 00'' to 16^0 29' 54'' North and longitude range of 73^0 54' 00'' East, with the altitude range of 1033 msl to 530 msl. It is a wildlife sanctuary covers an area about 251.16 sq. km. Itis well known for its floral and faunal diversity and one of the popular tourism point of the Western Ghats.In the present study insect herbivores, their distribution and host range in the forest was explored for two consecutive years. The survey was done during three seasons at day and night time.During the study, 46 plant species from 26 families for the pest incidenceswere observed. The insect herbivores were recorded 86 species of insects belonging to the orders Isoptera, Hemiptera, Thysanoptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera distributed within 31 families.

Key words:-Radhanagari forest, insect herbivores, distribution, host range

INTRODUCTION

The trees and insects have close associations with each other.Trees are the producers and insects are primary consumers. The order Coleoptera ranked first in maximum number of

species followed by Lepidoptera, Hymenoptera and Diptera. These four orders contribute about 80% in the entire class Insecta (Grimaldi and Engel, 2005).

Forest is a greatest storehouse of plant and animal species capable of providing many useful products. These are the centersfor variety of numerous present day and future crop plants and valuable timber. It maintains climate, soil, hydrological regime, biodiversity, the global carbon balance and overall security of human community (Puri, 1960; Shiva *et al*., 1992).

On contrary to traditional information tropical forests are not free of pest outbreaks. The Forests of Kolhapur are a southward extension of the forest types of Pune and Satara districts. The wooded areas are confined to the western half of the tract. The western rim and its descending slopes claim a stunted type of evergreen vegetation. On the eastern fringes the over-wood consists of deciduous species with a ground-flora of evergreen species. The dry eastern plain maintains scanty patches of wood-growth in land pockets. Three main forest types can be clearly located viz. the subtropical evergreen, the semi evergreen, the moist deciduous and the dry deciduous forest. Consumption of a plant material is a subject of great economic importance as well as biological importance (Barbosa and Schultz, 1987; Price *et al*., 1991; Hunter *et al*., 1992; Bernays and Chapman, 1994).

The other minor forest products are kaju fruits, watsol, cocumbs, mango fruit, bibi fruits, shikekai, palas leaves, kuchala seeds, kumkumfal, silver cotton, honey and wax, karanj seeds, rameta bark, Reeds, wavding, tembhurni leaves, pisa fruits. There are 134 chief trees species are found in the forested area of Kolhapur district. For instance*Terminalia tomentosa, Terminalia arjuna, Mangiferaindica, Careyaarborea, Cinnamomumnitidum, Terminalia bellirica, Syzygiumcumini, Lagerstroemia indica, Ficusracemosa, Leeaindica, Carissa carandas, Piper nigrum, Memecylonumbellatum, Zizyphusrugosa, Butea monosperma,Nothapodytesnimmoniana, Emblicaofficinalis, Cassia fistula, Lagerstroemia parviflora* are common trees species in forests of Kolhapur (www.maharashtra.gov.).

At present no confined information is available on the detailed insect plant interaction in the forest community. Researches are only focused on enlisting of insect pest on crop plants. In the present investigation an attempt has been made to enumerate the insect diseases on forest and plantation crop plants.

STUDY AREA

Radhanagari Wildlife Sanctuary is declared as Wildlife Sanctuary by G.R. No. WLP-1085.CR-588/V/F-5 of Government of Maharashtra, on 16th September, 1985. The Wildlife

Sanctuary spreads along the hilly terrain of the Sahyadri ranges of Western Ghats, located in Radhanagari Tehsil of Kolhapur District, Maharashtra. It is just 55 km away from Kolhapur. The sources of Bhogavati and Dudhganga rivers are located within, along with the entire catchments of Dudhganga and Radhanagari reservoir. It lies within the latitude range of 16^0 15' 00'' to 16^0 29' 54'' North and longitude range of 73^0 54' 00'' East, with the altitude range of 1033 msl to 530 msl. Radhanagari Wildlife Sanctuary covers an area about 251.16 sq. km. It is further categorized in as 115.73 sq. km core zone, 21.09 sq. km tourism zone, 96.03 sq. km developmental zone, 49.50 sq. km. eco-restoration zone with rest of malki land. It receives the huge rainfall during monsoon. The average range of rainfall is 2846 to 5520 mm (Pandeand Pathak, 2005). The temperature variation noted is about 4^0C to 41^0C.

METHODOLOGY

Extensive surveys were carried to find out insect herbivore complex during present study. The sampling sites were considered from the main forest types and sampling were done at each station in morning (07.00am to 11.00 am) and evening (05.00pm to 8.00pm). During the sampling, insect habitats on and within the trees and their associated insect groups were observed and sampled accordingly. The identification of insect specimens was made with the help of available literature (Frey, 1971; Mani, 1974; Mathur, 1975, Leather, 2005,).).

The field surveys were conducted during the years 2016 to 2018. The sampling was carried out during all the seasons. However, rainy season, the period from June to September found to be most suitable for collections, observations and to understand the association of insect herbivores within study sites. Because several insects start their activity or life cycle with the first downpour of monsoon.

Killing, preservation, mounting and labeling

The preservation, mounting and labeling of insect was followed as per Alfred and Ramkrishna (2004).

RESULT AND DISCUSSION

This study was an attempt to assess the present insect pests of indigenous material in the virgin forests. The survey and collection of insect pests was carried out at all the sites of Radhanagari Wildlife Sanctuary.

During the study, 46 plant species were observedfrom 26 families for the pest incidences (Table 1). Investigation on the insect herbivores recorded86 species of insects belonging to the orders Isoptera, Hemiptera, Thysanoptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera distributed within 31 families. (Table 2).

Not only insect show high diversity among all animals, it also reflected in their habitat and feeding habits. Associations of insect pests with specific plant organs were also recorded. They were found on flowers, fruits, foliage, stem and roots. Among these folivorous insects dominated the remaining groups.

Isoptera

Isopterans are mainly tropical in distribution. The low temperatures and high aridity are the limiting factors for termite survival. Very few species occur beyond 45^0 latitude (Collins, 1989). Observations on the termite infestation revealed that 10 different plant species found to be highly susceptible to termite attack within all the sites *viz.Terminalia arjuna, Terminalia tomentosa, Careyaarborea, Brideliaretusa, Mangiferaindica, Syzygiumcumini, Ficusracemosa, Eucalyptus globulus, Cinnamomumzeylanicum* and *Butea monosperma.* Felled trees were also found infested with termite. Initially, on forest trees, external runways covered with mud become evident and there are few internal galleries.

Hemiptera

Hemiptera include bugs that can be distinguished in two main groups/ suborders *viz.*Heteroptera and Homoptera comprising around 90,000 species (Grimaldi and Engel, 2005; Gillot, 1982). Homopterans are strictly phytophagusforms which are injurious to various cultivated plants. The major families of importance are Coccidae, Cicadidae, Psyllidae and Tingidae, Aleyrodidae, Aphididae (Mani, 1968, Nair, 2007). During the present study four families of hemipteran sap suckers were recorded *viz.*Psyllidae, Aleyrodidae, Aphididae, Cicadidae.

Psyllids are small plant feeding insects. These are host specific i.e. they only feed on one plant species or feed on few related plants.

They breed continuously during the growing season so long as new buds or foliage are available. Many species cause plant deformities in the form of regular galls, blisters or pits on the leaves or stem. Psyllids colonize also with aphids, phylloxerans, scale insects and white flies. They form the group called Sternorrhynka which is considered to be a most primitive group within the true bugs (Hemiptera).

Aphididae:-Collected on the leaves of *Mangiferaindica*

The order Thysanoptera includes comparatively minute insects popularly known as thrips. They have received better attention particularly due to their importance as pest of various agricultural crops and also because of their ability to act as vectors of some bacterial, fungal and virus disease of plants. For instance*Taeniothripsdistalis* and *Thripsflorum*are exclusively flower inhabiting while *Rhipiphorothripscruentatus* and *Retithripssyriacus* are always leaf inhabiting. Based on the habitat, thripsmay be grouped as Anthophilous, Phyllophilous, Phlaeophilous, Poephilous and Cecidogenous (Shull, 1914; Waston, 1926)

Lepidoptera

There are several pests of forests classified in the order Lepidoptera. But all are the pests of living trees; none are injurious to felled timber and very few destroy forest products other than wood (Beeson, 1941).

Pyralidae

Caterpillars feed on the foliage of *Tectonagrandis.* The species is active during August to November. In case of severe infestations, the entire set of plantation is skeletonized.

*Eutectonamachaeralis*is commonly known as 'Teak Leaf Skeletonizer.' It has narrow host range other than the *Tectonagrandis.* It has been recorded only on *Tectonahamiltoniana* and three species of *Callicarpa* viz. *C. arborea, C. cana* and *C. microphylla.*

Family Lymantridae

Caterpillars feed on the leaves of *Tectonagrandis,Syzygiumcumini and Terminalia bellirica..* The species is active during the months Oct to Dec.

Leaf Feeders

Leaf feeders include large proportion of forest insects distributed within the orders Coleoptera, Lepidoptera, Hymenoptera, Orthoptera and Diptera. Insects display different types of foliage feeding, according to their taxonomic groups. This leaf consumption can be divided in two types that are external leaf feeding or damage and internal leaf feeding (Regupathy*et al*., 1995).

Internal Leaf Damage

Internal leaf damage is usually known as leaf mining. In leaf mining, insects feed on the epidermal layers of leaf. Leaf miner insect does not feed in a single way throughout its life cycle that is early stages of few species are serpentine miners and late stages are blotch miners. Leaf mining is further classified into six types *viz.* linear mine. serpentine mine, blotch mine, Digitate mine, any combination of these, and needle mine.

Linear Mine

During the present study the linear mine was observed on the leaves of *Dendrocalamus* sp. at Radhanagari Wildlife Sanctuary within the study sites. The caterpillar prepared a distinct linear mine that adjoins and follows the secondary to primary veination closely. Regupathy*et al*., (1995) has given example of linear mine on Bamboo caused by *Cosmopteryxbambusae.*

Serpentine Mine

This type of mine was observed on the leaves of *Terminalia tomentosa* and *Terminalia arjuna.* It is usually tightly folded mine consisting at least four discernible phases. *Phyllocnistisamydropa* feed on the leaves of*Gmelinaarborea* which finally shows a picture of serpentine mine.

Blotch Mine

Roughly circular mined areas were observed on the foliage of *Anacardiumocidentale, Terminalia arjuna, Terminalia tomentosa, Syzygiumcumini, Tamarindusindica* and *Mangiferaindica.* For instance Caterpillars of *Acrocercopsphaeosphora* forms a blisher like mine at the central portion on the leaves of *Syzygiumcumini.* Severe infestation has been observed in hot weather.

External Leaf Damage

Regupathy*et al.*, (1995) mentioned four subtypes of external leaf damage *viz.* free feeding, Hole feeding, skeletonization and window feeding.

Free feeding

Insects feed greedily on the foliage leaving larger veins behind. Several lepidopterous caterpillars feed in this way. Examples are caterpillars of *Orgyiapostica*feed voraciously on the leaves of *Tectonagrandis* leaving only larger veins, *Trabalavishnu* in *Terminalia tomentosa*and *Metanastriahyrtaca* in*Syzygiumcumini.* Nair (2007) reported this type of feeding in *Hyblaeapuera.*

Hole Feeding

During the present study, this type of feeding was observed on several plants. Perforations of various sizes were found on foliage.

Skeletonization

Insects feed on the green content between the veins leaving veins as a skeleton. Developing stages of *Eutectonamachaeralis* and many lepidopterous insects feed on the soft material between the networks of veins which results into skeletonization of leaves.

Window Feeding

Early stages of many lepidpterous insects feed on the surface of the leaf. Caterpillars of *Thiacidaspostica* feed on the surface of *Z. jujuba* leaves. Surface abrasions were recorded on the leaves of several plants during the study..

Shelter Feeding

Thistype of feeding includes web enclosed feeding, leaf tying, leaf rolling, crinkled leaves, leaf and petiole galls. Several lepidopterous caterpillars web foliage and feed on the enclosed foliage. They may be surface feeders, skeletonizers or free feeders. The leaf rolling was observed on the leaves of *Dendrocalamus* sp., *Holorrhenaantidysentrica, Brideliaretusa, Ficusracemosa* at the study region.

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Table 1. List of plant species for insect herbivore incidence

Sr. No.	Scientific Number	Common Name	Family	Uses/ Importance
1	*Mangiferaindica*L.	Mango	Anacardiaceae	Fruits, Medicinal plant
2	*Holigarnagrahamii* (Wt.) Kurz.		Anacardiaceae	Medicinal plant
3	*Nothopegiacastaneifolia* (Roth) Ding Hong		Anacardiaceae	Fruits edible
4	*Ancistrocladusheneanus*Wall ex J. Graham		Ancistrocladaceae	Endemic to Western Ghats
5	*Carissa carandas* L.	Karvand	Apocynaceae	Fruits edible, Medicinal plant
6	*Mammeasuriga* (Buch.- Ham. ex. Roxb.) Kosterm	Surangi	Clusiaceae	Medicinal Plant
7	*Terminalia tomentosa*W& A	Ain	Combretaceae	Wood, Medicinal plant
8	*Terminalia arjuna* W&A	Arjun	Combretaceae	Wood, Medicinal plant
9	*Terminalia bellirica* (Gaertn)Roxb.	Behada	Combretaceae	Wood, Medicinal plant
10	*Aporosalindleyana* (Wt.)Bail.	Kebella	Euphorbiaceae	Endemic to Western Ghats
11	*Holorrhena antidysentrica* (Roxb. ex. Fleming) Wallich. ex. A. DC.	Eatser Tree	Euphorbiaceae	Medicinal plant
12	*Sapium insegne* Benth.	Hurra	Euphorbiaceae	Used to make detergent
13	*Albizzia lebbeck* (L.)Benth.	Libbeck	Fabaceae	Forage, medicine and wood
14	*Albizzia procera* (Roxb.) Benth.	Brown Albizia	Fabaceae	Often planted shade or beautification along road.
15	*Acacia auriculiformis* A.Cunn. ex. Benth.	Earleaf Acacia	Fabaceae	Used as firewood, timber, used as
16	*Garcinia indica*Choisy	Kokum	Guttiferae	Spices
17	*Nothapodytes nimmoniana*	Narkya	Icacinaceae	Medicinal plant
18	*Cinnamomumnitidum* Blume.	Indian Cassia	Lauraceae	Spices
19	*Cinnamomumzeylanicum*Breyn.	Tamalpatra	Lauraceae	Spices
20	*Litsea* sp.		Lauraceae	Wood
21	*Bielschmiediadalzel*		Lauraceae	Spices
22	*Careyaarborea*Roxb.	Kumbhi	Lecythidaceae	Wood, medicinal plant
23	*Pongamiapinnata* (L.) Pierre	Indian Beech	Leguminoceae	Cultivated for ornamental purpose and as a host plant for lac insect
24	*Dalbergialatifolia*Roxb.	Black Rosewood	Leguminoceae	Wood, in agro forestry, medicinal plant
25	*Cassia fistula* L.	Golden Shower	Leguminoceae	Ornamental, Medicinal plant
26	*Memecylonumbellatum*Burm.f.	Ironwoof	Melastomaceae	Medicinal Plant
27	*Ficusracemosa*Roxb.	Goolar Fig	Moraceae	Keystone species
28	*Ficushispida*L.f.	Hairy Fig	Moraceae	Keystone species
29	*Ficusvirens*L.	White Fig	Moraceae	Keystone species
30	*Knemaattenuata*(Hook.f. & Th.) Warb		Myristicaceae	Spices
31	*Myristicamalabarica*Lam.		Myristicaceae	Spices
32	*Eucalyptus globulus*Labill.	Blue Gum	Myrtaceae	Used as Wind breaker, Fuel wood , pulpwood

33	*Eucalyptus citriodora* Hook		Myrtaceae	Timber, Antimicrobial
34	*Syzygiumcumini*(L.)Skeels	Black Plum	Myrtaceae	Wood, medicinal plant, fruits edible
35	*Syzygiumlaetum* (Buch.-Ham.)Gandhi	Dev Jambhul	Myrtaceae	Medicinal plant, Endemic southern Western ghats
36	*Bridelia retusa* Spreng.	Katak	Phyllanthaceae	Medicinal Plant
37	*Piper nigrum*L.	Black Pepper	Piperaceae	Spices
38	*Zizyphusjujuba* Lamarck	Ber	Rhamnaceae	Fruiits edible, Medicinal plant
39	*Caralliabrachiata* (Lour.) Merr.	Freshwater Mangrove	Rhizophoraceae	Wood, Medicinal, ornamental plant
40	*Ixoranigricans* R. Br. Wt. &Arn.		Rubiaceae	Ornamental
41	*Pavettaindica* L.	Indian Pavetta	Rubiaceae	Medicinal plant
42	*Murrayakoenigi* (L.)Spreng.	Curry tree	Rutaceae	Spices
43	*Dimocarpuslongan*Lour.	Longan	Sapindaceae	Fruits edible, Medicinal plant.
44	*Grewia sp.*		Tiliaceae	Fodder
45	*Tectonagrandis*L.f.	Teak	Verbinaceae	Medicinal plant, wood
46	*Leea indica* (Burm.f.)Merr.	Bandicoot Berry	Vitaceae	Fodder

Table 2 List of insect herbivores

sr. No	Scientific Name	Order	Family	Common Name	Principal trees attacked	Affected plant parts
1	*Toxopteraoedinae?*	Hemiptera	Ahididae	Aphid	*Mangiferaindica*	Leaf
2	Unidentified	Hemiptera	Aleyrodidae	White fly	*Cassia fistula*	Leaf
3	Unidentified	Hemiptera	Aleyrodidae	White fly	*Oleadioica*	Leaf
86	*Xylocopoa aestuans* Linn.	Hymenoptera	Anthrophoridae	Carpenter Bee	*Ficus racemosa*	Branch
4	*Toxoptera*sp.	Hemiptera	Aphididae	Aphid	*Memecylonumbellatum*	Leaf
5	*Sinoxylon anale* Lesne ?	Coleoptera	Bostrichidae	False Powder Post Beetle	*Careya arborea*	Stem
6	*Psiloptera orientalis* Fab.	Coleoptera	Buprestidae	-	*Syzygium cumini, Acacia* sp	Bark
7	*Sternocera leavigata* Olivier	Coleoptera	Buprestidae	-	*Terminalia tomentosa, Terminalia arjuna*	Leaf
8	*Sternocera orientalis* Herbs	Coleoptera	Buprestidae	-	*Butea monosperma, Acacia catechu, Terminalia tomentosa*	Leaf
9	*Batocera rufomaculata* De Geer	Coleoptera	Cerambycidae	-	*Mangifera indica*	Stem
10	*Batocera*	Coleoptera	Cerambycida	-	*Mangifera indica*	Stem

	numitor Newman		e			
11	*Celosterna scabrator* Fab.	Coleoptera	Cerambycidae	-	*Pithocolabium dulce, Acacia nilotica*	Stem
12	*Coptops aedificator* Fab.	Coleoptera	Cerambycidae	-	-	-
13	*Olenocamptus bilobus* Fab.	Coleoptera	Cerambycidae	-	*Ficus racemosa*	Branches
14	*Acalolepta nivosa* White	Coleoptera	Cerambycidae	-	Dead wood	Wood
15	*Pterolophia sp*	Coleoptera	Cerambycidae	-	*Terminalia paniculata*	Branches
16	*Stibara nigricornis* Fab.	Coleoptera	Cerambycidae			-
17	*Glenea multiguttata* Guerin-Meneville	Coleoptera	Cerambycidae	-	*Mangifera iindica*	Branches
18	*Thylactus angularis* Pascoe	Coleoptera	Cerambycidae	-	-	-
19	*Xystocera globosa* Olivier	Coleoptera	Cerambycidae	-	*Albizzia lebbeck*	Stem
20	*Xylotrechus subcutellatus* Chever	Coleoptera	Cerambycidae	-	*Tectona grandis*	Stem
21	*Nyphasia apicalis*Gahan	Coleoptera	Cerambycidae	-	Dead wood	Wood
22	*Stromatium barbatum* Fab.	Coleoptera	Cerambycidae	-	-	-
23	*Priotyrannus mordax* White	Coleoptera	Cerambycidae	-	Dead rotten wood	Wood
24	*Aphrodisium cantori* Hope	Coleoptera	Cerambycidae	-	-	-
25	*Perissus sp.*	Coleoptera	Cerambycidae	-	*Carissa carandas*	
26	*Aeolesthes holosericea* Fab	Coleoptera	Cerambycidae	Stem Borer	*Terminalia tometosa, Terminalia arjuna, Albizia lebbeck, Albizia procera, Bridelia retusa*	Stem
27	*Platypriya andrewesi* Weise	Coleoptera	Chrysomelidae	Hispa	*Zizyphus jujuba*	Leaf
28	*Calopepla leayana* Latr.	Coleoptera	Chrysomelidae	--	*Gmelina arborea*	Leaf
29	*Clytrasoma palliatum* Fab.	Coeloptera	Chrysomelidae	--	*Glochidion sp.*	Leaf
30	*Monolepta signata* Olivier	Coeloptera	Chrysomelidae		*Zizyphus jujuba*	Leaf
31	*Curculio sp ?*	Coleoptera	Curculionidae	Weevil	*Careya arborea*	Leaf
80	*Myllocerus undecimpustulatus* Faust	Coeloptera	Curculionidae	Weevil	*Tectona grandis, Terminalia tomentosa, Terminalia arjuna*	

					Careya arborea	
81	*Apoderus tranquebaricus* Fab.	Coleoptera	Curculionidae	Giraffe Weevil	*Terminalia tomentosa, Terminalia arjuna, Mangifera indica, Dimocarpus longan, Mammea suriga, Lagerstroemia, Aporosa lindleyana, Syzygium cumini, Anacardium occidentale, Grewia sp.*	Leaf
82	*Balaninus- C-album* Schoenherr	Coleoptera	Curculionidae	-	*Syzygium cumini*	Fruits
83	*Deporaus marginatus* Pascoe	Coleoptera	Curculionidae	-	*Mangifera indica*	Leaf
84	*Cyrtotrachylus dux* Boheman	Coleoptera	Curculionidae	Bamboo Weevil	*Dendrocalamushamiltonii*and other species of Bamboo.	Young Sprouting Culm
32	*Argyroploce mormopa* Meyr.	Lepidoptera	Eucosmidae		*Syzygium cumini*	Leaf
85	*Leptocybe invasa* Fisher & La Salle	Hymenoptera	Eulophidae	Blue Gum Chalcid	*Eucalyptus globulus, Eucalyptus citriodora*	Leaf
33	*Dysphania percota* Swinhoe	Lepidoptera	Geometridae	Blue Tiger Moth	*Carallia brachiata*	Leaf
34	*Acrocercops phaeospora* Meyrick	Lepidoptera	Gracillaridae	Leaf miner	*Syzygium cumini*	Leaf
35	*Acrocercops syngramma* Meyrick	Lepidoptera	Gracillaridae	Leaf miner	*Anacardium occidentale*	Leaf
36	*Inderbela*sp	Lepidoptera	Inderbelidae	Bark Eating Caterpillar	*Syzygium cumini, Eucalyptus, Terminalia tomentosa, Terminalia arjuna*	Leaf
37	*Metanastriahyrtaca* Cramer	Lepidoptera	Lasiocampidae	Diamond back Caterpillar	*Brideliaretusa, Terminalia tomentosa, Syzygiumcumini, Terminaliaarjuna*	Leaf
38	*Trabalavishnu* Lefebure	Lepidoptera	Lasiocampidae	Lappet moth	*Terminaliaarjuna, Terminalia tomentosa, Syzygiumcumini, Buteamonosperma,Eucalyptusglobulus, Lagerstroemiasp.*	Leaf
39	*Parasalepida* Cramer	Lepidoptera	Limacodidae	Blue Striped Nettle Grub	*Careyaarborea, Butea monosperma, Brideliaretusa, Sapiuminsegne, Acacia auriculiformis, Terminaliaarjuna, Terminalia tomentosa, Mangifera indica, Syzygiumcumini.*	Leaf
40	*Natada velutina*	Lepidoptera	Limacodidae	-	*Terminalia tomentosa,*	Leaf

	Koll.				*Terminalia arjuna, Sapium insegne, Careya arborea, Mangifera indica*	
41	*Jamidesceleno* Walker	Lepidoptera	Lycaenidae	Common Cerulean	*Butea monosperma*	Leaf
42	*Orgyiaposticа*Walker	Lepidoptera	Lymantridae	Tussock Moth	*Tectonagrandis*	Leaf
43	*Orgyiaaustralis* Walker	Lepidoptera	Lymantridae	Tussock Moth	*Syzygiumcumini, Terminalia bellirica*	Leaf
44	*Carpophilus sp.*	Coleoptera	Nitidulidae	Sap Beetle	*Careya arborea*	Seed
45	*Spodopteralitur a*Fab.	Lepidoptera	Noctuidae	Cutworm	*Tectonagrandis, Mangiferaindica*	Leaf
46	*Carea angulata* Fab.	Lepiodoptera	Noctuidae	Nolid moth	*Syzygium cumini*	Leaf
47	*Asotaplana* Walker	Lepidoptera	Noctuidae	-	*Ficusracemosa*	Leaf
48	*Bombotelia delatrix* Guenee	Lepidoptera	Noctuidae		*Mangifera indica*	Leaf
49	*Thiacidaspostic a* Walker	Lepidoptera	Noctuidae	Ber white Hairy Caterpilla r	*Zizyphus jujuba*	Leaf
50	*Euthaliaaconthe asuddhodana*Fr ushthorper	Lepidoptera	Nymphalida e	Common Baron	*Mangiferaindica*	Leaf
51	*Crotonothrips sp.*	Thysanoptera	Phlaeothripd ae	Thrips	*Memecylonumbellatu m*	Leaf
52	*Gynaikothrips sp.*	Thysanopotera	Phlaeothripd ae	Thrips	*Ancistrocladushenean us*	Leaf
53	*Liothripskaryni* Bagnall	Thysanoptera	Phlaeothripd ae	Thrips	*Piper nigrum*	Leaf
54	*Gynaikothrips sp.*	Thysanoptera	Phlaeothripi dae	Thrips	*Terminalia arjuna, Terminalia tomentosa*	Leaf
55	*Austrothripscoc hinchinensis*Kar ny	Thysanoptera	Phlaeothripi dae	Thrips	*Calycopteris floribunda*	Axial Buds
56	*Catopsililapomo na* Fab.	Lepidoptera	Pieridae	Common Emigrant	*Cassia fistula*	Leaf
57	*Catopsiliapyran the* L.	Lepidoiptera	Pieridae	Mottled Emigrant	*Cassia fistula*	Leaf
58	*Triozafletcheri minor* Crawford	Hemiptera	Psyllidae	Psyllid	*Terminalia arjuna, Terminalia tomentosa*	Leaf
59	*Triozajambolan ae* Crawford	Hemiptera	Psyllidae	Psyllid	*Syzygiumcumini*	Leaf
60	*Pauropsylladepr essa* Crawford	Hemiptera	Psyllidae	Psyllid	*Ficusracemosa*	Leaf
61	*Pauropsylla sp.*	Hemiptera	Psyllidae	Psyllid	*Litsea* sp.	Leaf
62	*Eutectonamacha eralis* Walker	Lepidoptera	Pyralidae	Teak Skeletoni zer	*Tectonagrandis*	Leaf
63	*Antheraeamylitt a* Drury	Lepidopterqa	Saturniidae	Tasar Silk Moth	*Syzygium cumini, Terminalia tomentosa, Terminalia arjuna*	Leaf
64	*Actiasselene*Hub ner	Lepidopterqa	Saturniidae	Moon Moth	*Terminaliaarjuna, T. tomentosa, Ficus*	Leaf

					bengalensis.	
65	*Attacus atlas* Linn	Lepidopterqa	Saturniidae	Atlas Moth	*Sapiuminsegne, Holorrhenaantdidysentrica, Embeliaribes*	Leaf
66	*Brahmina sp.*	Coleoptera	Scaeabaeidae		*Cassia fistula*	Leaf
67.	*Holotrichia fissa* Brenske	Coleoptera	Scarabaeidae	Chafer beetle	*Emblica officinalis, Bridelia retusa, Careya arborea, Zizyphus jujuba, Butea monosperma, Grewia sp., Terminalia tometosa, Terminalia arjuna, Syzygium cumini*	Leaf
68	*Holotrichia karschi* Brenske	Coleoptera	Scarabaeidae	Chafer beetle	*Syzygium cumini, Bridelia retusa, Acacia auriculiformis, Terminalia T. arjuna, T. tomentosa*	Leaf
69	*Adoretusversutus* Harold	Coleoptera	Scarabaeidae		*Zizyphus jujuba, Terminalia tomentosa, Terminalia arjuna*	Leaf
70	*Adoretuslasiopygus* Burm.	Coleioptera	Scarabaeidae		*Terminalia arjuna*	Leaf
71	*Maladeracastanea* Arrow	Coleioptera	Scarabaeidae		*Carissa carandas*	Leaf
72	*Maladeraholosericea* Scopoli	Coleioptera	Scarabaeidae		*Carissa carandas*	Leaf
73	*Apogonia* sp	Coleoptera	Scarabaeidae		*Carissa carandas, Tectona grandis*	Leaf
74	*Clinteria sp.*	Coleoptera	Scarabaeidae		*Mimusops elengi*	Flowers
75	*Acherontiastyx* Westwood	Lepidoptera	Sphingidae	Deaths Head Hawk Moth	*Tectonagrandis*	Leaf
76	*Psilogrammamenephron* Cramer	Lepidoptera	Sphingidae	Hawk Moth	*Tectonagrandis*	Leaf
77	*Odontotermesobesus*Rambur ?	Isoptera	Termitidae	White Ant	*Careyaarborea, Mangifera indica, Terminalia tomentosa, Terminaliaarjuna, Nothapodytesnimmoniana, Brideliaretusa*	Stem
78	*Rhipiphorothripscruentatus*Hood	Thysanoptera	Thripidae	Thrips	*Careyaarborea*	Leaf
79	*Eterusia virscens* Butler	Lepidoptera	Zygaenidae	-	*Sapium insegne*	

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Research Article
Allelopathic Effect of *Excoecaria agallocha* L. Mangrove Leaf Leachate on Germination and Growth Behavior of *Eleusine coracana* (Finger Millet)

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Abstract

Background and Objective: Allelochemicals extracted from plant either through leaf or root can affect growth and productivity of surrounding plants and crops. The purpose of this study was to determine the morphological, physiological and yield responses of finger millet growing near the mangrove *Excoecaria agallocha* L. in coastal areas. **Materials and Methods:** The study was conducted in the laboratory from November, 2016 to January, 2017 and used Randomized Block Design (RBD) with two factors, different concentrations (25, 50, 75 and 100%) of leaf leachates of mangrove *Excoecaria agallocha* and two finger millet varieties (brown and white). Germination, shoot length, seedling dry matter, vigour index were recorded at 10 days after sowing in all the test crops. The photosynthetic pigments, soluble proteins, osmolyte proline and phenol content were also estimated. Further gas chromatographic analysis was also done for the identification of allelochemicals. Correlation coefficient was determined by plotting data from all treatments and the relation amongst seedling growth parameters and seedling vigour index was examined. Significance between control and treatment was compared at 0.05 probability level. **Results:** Results showed that *E. agallocha* leaf leachate extracts have significant effect ($p<0.001$) on the entire seedling growth and highest inhibition was observed at 75 and 100% concentration. The correlation study revealed that the vigour index was significantly correlated with shoot length ($p<0.05$) and dry matter ($p<0.01$) for 100% leaf litter leachate concentration **Conclusion:** It is concluded that the inhibitory effect of the test species on seed germination and seedlings of finger millet cultivars may be related to the presence of allelochemicals including fatty acids, flavonoids and phenolic acids.

Key words: Crop physiology, physiological attributes, growth, development, allelopathic response, *Excoecaria agallocha,* finger millet

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Allelopathy is fascinating and perplexing subject that concerns with the interaction of plants as influenced by the chemical substances that they release into the environment[1-2]. Allelopathy is a mechanism in which chemicals produced by some plant species may increase or decrease the associated plant growth[3]. Such positive or negative effects are due to release of active biomolecules commonly called as "Allelochemicals"[4]. Allelopathic effect of one plant on the growth and development of the same plant or neighboring plants is due to production of a large variety of secondary metabolites. Allelochemicals usually are secondary metabolites, which are produced as byproducts during different physiological processes in plants[5,6]. As a result of litter decomposition a huge number of phyto compounds gets released into the environment. The multiple effects resulting from allelochemicals include effect on cell division, production of plant hormones, membrane permeability, germination of pollen grains, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, protein synthesis, nitrogen fixation and specific enzyme activities[7,8]. Allelopathic compounds generally occur in natural plant communities and are suggested to be one mechanism by which weeds interfere with crop growth[9]. Several weed species are reported to have allelochemicals that affect germination and growth of crops due to toxicity[10]. The phytotoxic allelochemicals liberated from plants can exert stress which indirectly or directly affects the receiver plants[11]. Plant parts are the source of allelochemicals[12]. Large number of compounds are released by plants in their surroundings which are having either deleterious or beneficial role in an environment[13]. Allelopathy is expected to be an important mechanism in the plant invasion process because the lack of co-evolved tolerance of resistant vegetation to new chemicals produced by the invader could allow these newly arrived species to dominant natural plant communities[14].

Excoecaria agallocha L. is a member of Euphorbiaceae family also known as 'milky mangrove', 'blind-your-eye mangrove' and 'river poison tree'. Contact with skin can cause irritation and rapid blistering; contact with eyes will result in temporary blindness. This plant grows along the banks of cultivated field in konkan region of Maharashtra. Li *et al.*[15] studied allelopathic effect of *Sonneratia apetala* on a weed *Spartina alterniflora.* Allelopathic influence of *Excoecaria agallocha* L. on seed germination and seedling growth of some pulses and millets was studied by Kavitha *et al.*[16]. The widespread and persistent occurrence of this mangrove plant near coastal fields and especially around paddy and ragi crop fields makes it suspicious to cause some adverse effect on these crops through allelopathic interaction. Therefore there is always a threat that it may become a major problem of our cropping system. Keeping in view these facts, this study was planned to evaluate the phytotoxic effect of *E. agallocha* on germination, seedling growth, dry biomass, total chlorophyll content and biochemical parameters of *E. coracana*.

MATERIALS AND METHODS

Collection and preparation of aqueous leachate from leaf litter: Leaf litter of mangrove *E. agallocha* were collected from old trees growing on the banks of paddy fields in village Malvan, Konkan, Maharashtra. The study was conducted in the laboratory from November, 2016 to January, 2017 and used Randomized Block Design (RBD) with two factors, different concentrations (25, 50, 75 and 100%) of leaf leachates of mangrove *Excoecaria agallocha* and two finger millet varieties (brown and white). Samples were recorded by collecting recently fallen leaf litter (identified by its yellow or light brown color). The leaves were sun dried and cut into 3-6 cm pieces. The leaf litter leachates were prepared in our Laboratory, by soaking 200 g leaf litter in 200 mL double distilled water in a flask for 24 h at room temperature (32±2°C). The leaf litter leachates were filtered first through cheese cloth and then with Whatman No. 1 paper. The leaf litter leachate was diluted with distilled water to 25, 50 and 75 (v/v) concentrations and distilled water was used as control.

Bioassay: Twenty five seeds of each finger millet (*Eleusine coracana*) variety viz white and brown were chosen as test plant and pre-soaked overnight in distilled water. Before seed germination test, empty and undeveloped seeds were discarded by floating in tap water. The selected seeds of finger millet were thoroughly washed with tap water to remove dust for 5 min. To avoid possible inhibition caused by toxins from fungi or bacteria, seeds were surface sterilized with 10:1 distilled water/bleach (commercial NaClO) solution for 5 min and then washed 6-7 times with distilled water. Sixty seeds were divided into three replicates of 20 seeds each were soaked for 4 h in 25, 50, 75 and 100% of leaf leachates. Control seeds were soaked in 10 mL of distilled water. The seeds were allowed to germinate in 20 cm diameter petri dishes with a tight-fitting lid and placed in sterilized polythene bags to prevent further loss of volatiles and kept in a seed germinator and were irrigated with

Table 1: Effect of leaf leachates of *E. agallocha* on seed germination and seedling growth of finger millet cultivars at 10 days after sowing

Concentration of leaf leachates	Germination (%)		Shoot length (cm)		Root length (cm)		Dry matter (g plant^{-1})		Vigour index	
	White	Brown	White	Brown	White	Brown	White	Brown	White	Brown
Control (DW)	90	95	6.3	6.8	4.7	5.6	0.45	0.46	991.8	996.5
25%	85	75	5.6	5.1	3.9	4.6	0.39	0.40	596.3	624.8
50%	70	65	5.0	4.4	3.4	4.2	0.36	0.35	498.2	541.3
75%	60	55	3.8	3.9	2.9	3.8	0.32	0.33	365.7	404.5
100%	55	50	2.7	2.9	2.4	2.7	0.27	0.29	302.4	337.5
CD at 5%	1.6	1.4	0.12	0.16	0.12	0.14	0.02	0.02	56.9	57.8

Table 2: Pearson correlation of biochemical parameters of test plants

Leaf litter leachate concentration (%)	Parameters	Level of significance
50	Phenol and chlorophyll	p<0.001
75	Phenol and proline	p<0.01
100	Vigor index and shoot length	p<0.05
	Vigor index and dry matter	p<0.001
	Phenol and soluble sugars	p<0.01
	Vigor index and phenol	p<0.05

10 mL leachate on alternate days. Germination, shoot length, seedling dry matter, vigour index were recorded at 10 days after sowing in all the test crops.

Biochemical analysis: Biochemical parameters such as chlorophyll contents were estimated according to Aron[17], by extracting the chlorophyll in 80% acetone and expressed as mg g^{-1} FW. Soluble protein was extracted from the leaf samples of the test according to the method of Lowry *et al.*[18] and expressed as mg g^{-1} FW. Estimation of proline was done following the method of Bates *et al.*[19]. Proline was extracted in 3% sulfosalicylic acid, estimated by using acid ninhydrin reagent and measuring the absorbency of the toluene chromophore at 520 nm and expressed as µM g^{-1} DW. Total phenol content was assayed according to Swain and Hillis [20] and expressed as µg g^{-1} DW.

GC-MS analysis for phytochemical compounds: Samples were analysed with a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a Gerstel MPS2 auto sampler and coupled to a HP 5973 N mass spectrometer. The carrier gas was helium (BOC gases, Ultra High Purity), flow rate 1.2 mL min^{-1}. The oven temperature was started at 50°C, held at this temperature for 1 min, then increased to 220°C at 10° min^{-1} and held at this temperature for 10 min. The injector was held at 200°C and the transfer line at 250°C. For quantification of the compounds, mass spectra were recorded in the Selective Ion Monitoring (SIM) mode using NIST library.

Statistical analysis: Statistical analysis of data was done using software of SPSS 13.0. Nivas and Gaikwad[13] correlation coefficient was determined by pouting data from all treatments and the relation amongst seedling growth parameters and seedling vigour index was examined. Significance between control and treatment was compared at 0.05 probability levels.

RESULTS

Germination and seedling growth: The increase in leaf litter leachate concentrations reduced the seeds germination (%) (Table 1). The higher concentrations of leachate (75,100%) drastically reduced the germination . The germination in both accessions of finger millets were inhibited by leaf litter leachates over control and the inhibition followed the order: white>brown. The inhibition was more at higher concentration in white variety over control. Similar to germination, the shoot height in both finger millet accessions were inhibited due to increase in leaf litter leachate concentrations. The maximum suppression in shoot height was recorded in variety white variety at higher leachate concentrations, over control. Similarly in brown variety the highest decrease in shoot height was noticed at 100% leachate concentration. The roots of white and brown finger millet varieties appeared more sensitive to the *E. agallocha* leaf litter leachate and the suppression was pronounced over

control, which was further reflected in accumulation of dry matter at 100% concentration. The reduction was also recorded in vigour index, with the increasing leachate concentrations. The leaf litter leachate significantly (p<0.05) reduced the vigour index in all rice varieties. The highest reduction in vigour index was recorded in both varieties, with higher doses of leachate concentrations over control (Table 1). The correlation study revealed that the vigour index was significantly correlated with shoot length (p<0.05) and dry matter (p<0.01) for 100% leaf litter leachate concentration (Table 2).

Photosynthetic pigments and biochemical parameters: The chlorophyll contents were reduced significantly (p<0.05) in all treatments than control (Table 3). Moreover, the reduction of chlorophyll-b was greater than chlorophyll-a in both finger

Table 3: Effect of leaf leachates of *E. agallocha* on photosynthetic pigments of two finger millet cultivars at 10 days after sowing

	Chlorophyll a (mg g^{-1} FW)		Chlorophyll b (mg g^{-1} FW)		Total chlorophyll (mg g^{-1} FW)	
Concentration of leaf leachates	White	Brown	White	Brown	White	Brown
Control (DW)	1.52	1.63	0.94	0.95	2.61	2.68
25%	1.35	1.38	0.70	0.72	2.17	2.34
50%	1.27	1.30	0.64	0.65	1.97	1.96
75%	1.09	1.10	0.53	0.55	1.78	1.82
100%	0.82	0.93	0.48	0.50	1.59	1.63
CD at 5%	0.03	0.02	0.04	0.06	0.12	0.14

FW: Fresh weight, DW: Dry weight

Table 4: Effect of *E. agallocha* leaf leachates on soluble protein, proline and phenol content of two finger millet cultivars at 10 days after sowing

	Soluble protein (mg g^{-1} FW)		Proline (μM g^{-1} DW)		Phenol (μg g^{-1} DW)	
Concentration of Leaf leachates	White	Brown	White	Brown	White	Brown
Control (DW)	9.96	10.87	32.30	36.50	122.45	134.2
25%	7.65	8.65	40.12	46.25	137.20	149.3
50%	6.13	6.84	58.32	63.78	225.80	246.3
75%	5.73	5.96	60.20	72.60	379.80	385.7
100%	4.29	5.12	68.70	83.74	409.10	412.6
CD at 5%	0.43	0.32	1.80	2.30	2.10	1.8

DW: Dry weight, FW: Fresh weight

Table 5: Chemical composition of leaf extract

Compounds	Composition (%)
Stigmasterol	2.6
β-sitosterol	3.1
Lupeol	7.8
Phytol	23.4
Squalene	3.2
Stigma 4 en 3 one	8.3
9,12-octadecadienoic acid (z,z) octyl ester	1.6
1,2,3-benzenetriol	3.1
Lauric acid	16.32
Myristic acid	7.81
Palmitic acid	23.8
Arachidic acid	2.7
Behenic acid	0.82
Oleic acid	2.7
Stearic acid	1.3
Linoleic acid	2.1

millet test varieties at higher leachate concentrations (75 and 100%). The decrease in the chlorophyll concentration of finger millet varieties in all concentration of leachate further reflects the reduction or decrease in the total soluble sugar. In this study maximum reduction of total sugar was observe at 100% leachate concentration in all three rice varieties over control (Table 4). The free proline content in seedlings of both varieties increased with increasing leachate concentrations (Table 4). About 68% of proline was increased over control by 100% leachate concentration in brown variety. The phenol content in both finger millet seedling increased with increasing leachates concentrations than control (Table 4). The correlation study revealed that vigour index positively related ($p<0.05$) with phenol content at 100% leachates concentration (Table 2).

Phytochemical analysis: Mass spectral analysis of bioactive volatile compounds of *E. agallocha* leaves is shown in Table 5. It is clear from the table that the leaf leachate is rich in bioactive compounds. The major compound stigma 4 en 3 one and 9,12-octadecadienoic acid (z,z) octyl ester, squalene while other compounds are stigmasterol, β-sitosterol, 1,2,3-benzenetriol and phytol.

DISCUSSION

Among the concentrations, maximum inhibition was observed in 100%, followed by 75%. Similar observation in reduction of seed germination also reported in different crops[21]. An indirect relation between lower germination rate and allelopathic inhibition may be the result of inhibition of water uptake[22]. The inhibition of germination is dependent on the concentration of the extract; perhaps it may be due to the entry of water soluble allelochemicals into the seed, which retards the germination and growth[23]. Likewise, the results of present investigation on allelopathic effect of *E. agallocha* results are in accordance with previous studies of Nivas and Gaikwad[13] and Kavitha *et al.*[16] who observed allelopathic effect of *E. agallocha* on some pulses and millets. The allelopathic effect of different leaf leachate concentration on shoot length have been summarized in the Table 2. Shoot length was found to be suppressed significantly ($p<0.001$) in white variety with all treatments exhibiting concentration dependent. Nevertheless, the root length of both finger millet varieties were greatly inhibited with the increasing of concentration of leachates. The inhibitory effect was much more pronounced in brown variety

at higher concentration. Among the studied varieties white exhibited more sensitive response. The inhibition shoot length by *E. agallocha* may be due to the presence of some phenolic compounds. These phenolic compounds might have interfered with the phosphorylation pathway or inhibiting the activation of Mg and ATPase activity or might be due to decreased synthesis of total carbohydrates, proteins and nucleic acids (DNA and RNA) or interference in cell division, mineral uptake and biosynthetic processes[24]. Also, allelochemicals inhibit absorption of ions[25] hence, resulted in arrested growth[26]. The results obtained in the present study were correlate with Beres and Kazinczi[25] in field crops, Sasikumar *et al*.[26] in pulse crops and Kavitha *et al*.[16] in pulses and millets, who noticed that root and shoot growth was inversely correlated to the concentration of the leachate solution. In the present study also increase in concentration retarded the growth of both the root and shoot and eventually affecting the overall length of the seedling. Similarly, the vigour index was also reduced with corresponding increase in concentration. Djanaguiraman *et al*.[27] found a similar type of result, that *E. globules* reduced the vigour index in green gram, black gram and cowpea. A similar inhibitory effect of *Digera muricata* on *Sorghum* was reported by Karthiyayini *et al*.[28]. Rashid *et al*.[29], reported impaired growth of lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) seeds (root and shoot length and fresh weight) by the allelopathic potential of leaf and root leachates of kudzu (*Pueraria lobata*). Tanveer *et al*.[30] also reported that minimum GI and germination percentage of rice seeds were observed when such seeds were treated with leaf leachates of common cocklebur (*Xanthium strumarium)*. Sahoo *et al*.[31] reported the reduction in dry weight of chilli, soybean, maize, rice and lady's finger at higher concentrations of aqueous leaf extract from *Mangifera indica* L.

The reduction in vigour index in the studied Finger millet varieties may be due to reduced germination and shoot length as vigour index is the product of germination and seedling length. Dry matter per plant directly affects the final yield. *Excoecaria agallocha* leaf leachates decreased the dry matter in all the tested finger millet varieties and the magnitude of reduction was maximum in white variety. The magnitude of reduction in both varieties was concentration dependant. The reduction of biomass was correlated with reduced seedling growth. The reduction in biomass may be due to stunted and reduced seedlings growth[24]. These indicate, among the test varieties brown was comparatively tolerant to growth suppressed due to *E. agallocha* leaf leachates.

In white variety chlorophyll a and b was reduced by 50% and total chlorophyll by 70% in 100% concentrated leaf leachates. Reduction in photosynthetic pigments due to adverse effect allelochemical stress was previously reported[22]. The results of the present study are in accordance with the finding of Singh and Rao[32]. Siddiqui[33] reported reduction in chlorophyll content of *Vigna mungo* due to the allelochemicals present in leachate of black pepper which possibly target enzymes responsible for the conversion of porphyrin precursors. The reduction in chlorophyll contents observed in all the concentrations might be due to degradation of chlorophyll pigments or other phytochemicals present in leaf leachates[22]. The more reduction of chlorophyll b than chlorophyll a, indicates its susceptibility to stress[18]. Reduction in chlorophylls may decrease the photosynthesis and thereby substantially decrease further metabolites like total sugars, proteins and soluble amino acids[34]. Oyerinde *et al*.[35] revealed the decrease in chlorophyll a, chlorophyll b and total chlorophyll accumulation in young plants of maize after being treated with fresh shoot aqueous extract of *Tithonia diversifolia* which is a weed plant known to possess allelopathic characteristics. Hussain and Reigosa[36] reported similar results regarding the effects of allelochemicals on chlorophyll content and photosynthesis process in plants.

Highest accumulation of proline was observed in white var. (87.46 μM g^{-1} DW) in comparison to brown variety (83.74 μM g^{-1} DW) at higher concentration (100%) of leaf leachates. The similar type of result in increase in proline content in sorghum was reported by Pawar and Chavan[34] also observed. The proline accumulation under stress conditions is mainly due to increased synthesis from glutamate. Increased proteolysis in germinating seeds can lead to increase in free proline content along with other amino acid[34].

The maximum phenol content was observed in white variety (438.9 μg g^{-1} DW) at 100% concentration. Increase in phenol contents was also responsible for reducing the seedling growth. The increase in phenol contents was correlated with reduction in seed germination and seedling growth of white variety. The maximum phenol contents in concentrated leaf leachates (100%) suggested that the concentration of phytotoxic allelochemicals inhibitory to growth.

According to spectral data, the identified compounds; stigma 4 en 3 one and 9,12-octadecadienoic acid (z,z) octyl ester are active phyto components against pathogens. Xian *et al*.[37] reported several fatty acids with allelopathic activity. The myristic acid and palmitic acid inhibits the plant

growth[38]. Geethambigai and Prabhakaran[39] studied the allelopathic potential of *Cyperus* and *Cynodon* on germination and growth of some rice cultivars and found that the weed *C. dactylon* contains beta-sitosterol, beta-carotene, vitamin C, palmitic acid and triterpenoids. The allelochemicals present in the weed extracts might be jointly synergistic to seeds germination and seedling growth of finger millet. Similarly the phytotoxicity of *E. agallocha* in this study might be due to the interactions of various groups of fatty acids and phenols.

Taking this in to account, it is necessary to ascertain whether the results observed for fallen senescent leaves are same for recently fallen leaves and whether they vary with seasonal conditions. Experiments to investigate its allelopathic behavior on other crop plants and to better understand the role of this mangrove plant in structuring cropping in coastal areas.

CONCLUSION AND FUTURE RECOMMENDATIONS

The results suggest that *E. agallocha* leaf leachate extract contain secondary metabolites that exhibit allelopathic effects at early stages of growth of finger millet plants under laboratory conditions. The growth inhibitory effect was stronger. Hence, if present in field, this plant can disturb the stand establishment of crop plant. There is a need to take a serious notice of the presence of this plant in the crop fields and nearby places. Further research can explore the allelochemicals present in *E. agallocha* as well as the complex allelopathic mechanisms through which this phytotoxic plant disturbs the neighboring plants.

SIGNIFICANCE STATEMENTS

This study discovers the possible inhibitory effect of water soluble substances present within the leaves of mangrove species. This study will help the local inhabitant and researcher to understand the allelopathic mechanism of mangrove *E. agallocha* for the lowering of growth and productivity of crop plants. Further, potential allelochemicals must be characterized as they can provide new and cheap synthetic analogues of natural products having greater selectivity, stability and efficacy to control weeds and pests. They should also undergo toxicity testing to confirm their safety on non-target species.

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Research Article
Allelopathic Potentials of *Chromolaena odorata* L. on Growth and Biochemical Characteristics of *Salvadora persica*

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Abstract

Background and Objective: Siam weed is a fast-growing perennial, is now regarded as one of the most harmful weeds present on earth due to its highly invasive and allelopathic nature. The present investigation was aimed to evaluate the allelopathic effect of aqueous extract and leaf litter leachate extracts from *Chromolaena odorata* on the growth of mangrove associate plant *Salvadora persica*. **Materials and Methods:** Germination of *Salvodora persica* was performed in Petri dishes under different concentrations (20, 40, 60 and 80%) of leaf aqueous extracts of *Chromolaena odorata* collected from natural habitats of Konkan region of Maharashtra, India. At the end of experiment (10 days), the growth and biochemical characteristics of *S. persica* seedlings were measured. The data were statistically analyzed by one-way ANOVA analysis of variance. **Results:** The effects of extracts on germination percentage, seedling growth and dry biomass were investigated. Higher concentrations of extract (60 and 80%) significantly reduced germination percentage, radicle length, plumule length and dry matter accumulation of the *Salvadora* seedlings as compared to control. The inhibitory effect was concentration dependent and the more pronounced effect noticed in leaf litter leachate extract. A significant correlation was found in different concentrations of extracts used with respect to morphological attributes. The allelopathic effect also noticed in biochemical parameters. The GC MS analysis of leaves revealed the presence of some allelochemical compounds which supported the allelopathic potential of leaf extract. **Conclusion:** Based on the findings, it could be speculated that the delayed germination and low germination rate of the test species after treatment by extracts may be due to the allelochemicals present in the extracts which might release phenolics into the soil and these are probably involved in the growth inhibitory effect of surrounding plant species.

Key words: Fast growing perennial, allelopathic potential, siam weed, biochemical changes, harmful weeds, *Chromolaena odorata*, *Salvadora persica*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Allelopathy is a natural biological phenomenon in which an organism produces some biochemical compounds which influence the growth, survival, reproduction and biological processes of other organisms. These biochemical compounds are known as allelochemicals and can have positive allelopathy (beneficial) or negative allelopathy (detrimental) effects on the targeted organisms. According to Stamp[1], these allelo-chemicals are a set of secondary metabolites, which are not required for metabolic activities such as growth, development and reproduction of the mother organism. These effects are selective and concentration dependent and may have an either inhibitory or stimulatory effect on the growth of subsequent crops or weeds[2-4]. A Large number of chemicals have been identified and found to release from crops through volatilization, leaching, decomposition of crop residues and root exudation. Recently, the focus of role of allelopathy in agriculture is shifted towards effects of leachates from plants, plant extracts and decomposing plant residues. Plant residues generally contain a variety of toxic compounds that are known inhibitors of seed germination or seedling development[5]. Plant leachates are recorded to have suppression of seed germination and vegetative propagules and also early seedling growth[6,7]; the decrease in radicle growth has also been reported by Casado[8]. According to Patrick[9], the degree of damage to the crop is related to the degree of contact of roots to the leachates or residues. Phyto-toxicity from a crop might be from indirect effects of micro-organisms and direct toxic actions[10]. Aqueous extract of some plants reduces seedling growth[11]; root and shoot growth[12]; germination[13] and stimulate mortality of plants[14].

Chromolaena odorata (Linn) R.M. King and H. Robinson (Asteraceae) (syn. *Eupatorium odoratum* L. or *Osmia odorata* L.) known as siam weed is a fast-growing perennial, diffuse and scrambling shrub native to central and southern America, then introduced into the tropical regions of Asia, Africa and the Pacific where it is an invasive weed. The plant grows to 3-7 m in height when grown in the open and it goes by many common names including devil weed, French weed, communist weed, hagonoy, co hoy etc.[15-19]. This invasive weed is introduced to many places, either intentionally as an ornamental plant or accidentally, it is now regarded as one of the most harmful weeds present on earth due to its highly invasive and allelopathic nature[17]. This weed suppresses crops and other plants in its surroundings by competing for nutrients and water, over-shading and allelopathy[20]. The younger leaves of *C. odorata* are toxic due to high levels of nitrate[21].

In a preliminary survey, it was marked that a vast area of land, especially in the roadside of the coastal area of Sawantwadi, Maharashtra are infested with the weed. The literature on the effect of siam weed or its extracts on the growth of other coastal plants is quite scanty. Keeping in mind the above background, the present study was undertaken with the following objectives: (i) To evaluate the allelopathic effects of aqueous extract of leaf extract and leaf litter leachate extract of siam weed on morphological and biochemical parameters, of a coastal plant *Salvadora persica*, (ii) Identification of allelo-chemicals through GC-MS analysis of both extract and (iii) To identify which extracts cause sufficient suppression to growth of the studied species.

MATERIALS AND METHODS

Preparation of aqueous extract: The fresh leaves of Insect-free, disease-free plants of *C. odorata* and fallen matured senescent leaves were collected from the old trees growing in the coastal area near Sawantwadi, Maharashtra, during the months of November-December, 2016 and further analysis was carried out in next 6 months. The leaves were washed thoroughly with distilled water and air-dried at room temperature for 96 h. Both, fresh as well as fallen matured leaves chopped into 1 cm long pieces and were grated with the mechanical grater. The ground plant was soaked in 1 L of water for 24 h. The extracts were then filtered with muslin cloth followed by Whatman filter paper No. 1. This served as the stock solution from which other concentrations (20, 40 and 60%) were prepared by way of dilution.

Bioassay: The seeds of a test plant *Salvadora persica* were first treated with distilled water to remove the stress of debris and sand particles. Before seed germination test, empty and undeveloped seeds were discarded by floating in tap water. To avoid possible inhibition caused by toxins from fungi or bacteria, seeds were surface sterilized with 10:1 distilled water/bleach (commercial NaClO) solution for 5 min and then washed 6-7 times with distilled water. Sixty seeds were divided into three replicates of 20 seeds each were soaked for 4 h in 20, 40, 60 and 80% of leaf extract and leaf litter leachate extract. Control seeds were soaked in 10 mL of distilled water. The seeds were allowed to germinate in 20 cm diameter Petri dishes with a tight-fitting lid and placed in sterilized polyethene bags to prevent further loss of volatiles and kept in a seed germinator and were irrigated with 10 mL of the extract on alternate days. The emergence of a radical approximately 1 mm in diameter was taken as the index of germination. The dry biomass was determined after oven drying at 80°C for 24 h.

Biochemical analysis: Biochemical parameters such as Chlorophylls content were estimated according to Arnon[22], by extracting the chlorophylls in 80% acetone and expressed as mg g^{-1} FW. Soluble protein was extracted from the leaf samples of the test according to the method of Lowry *et al.*[23] and expressed as mg g^{-1} FW. Estimation of proline was done following the method of Bates *et al.*[24]. Proline was extracted in 3% sulfosalicylic acid, estimated by using acid ninhydrin reagent and measuring the absorbency of the toluene chromophore at 520 nm and expressed as µM g^{-1} DW. Total phenol content was assayed according to Swain and Hillis[25] and expressed as µg g^{-1} DW.

GC-MS analysis for phytochemical compounds: Samples were analyzed with a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a Gerstel MPS2 autosampler and coupled to an HP 5973 N mass spectrometer. The carrier gas was helium (BOC gases, Ultra High Purity), flow rate 1.2 mL min^{-1}. The oven temperature was started at 50°C, held at this temperature for 1 min, then increased to 220°C at 10°C min^{-1} and held at this temperature for 10 min. The injector was held at 200°C and the transfer line at 250°C. For quantification of the compounds, mass spectra were recorded in the Selective Ion Monitoring (SIM) mode using NIST library.

Statistical analysis: Statistical analysis, one way ANOVA of data was done using software of SPSS 13.0. The correlation coefficient was determined by pouting data from all treatments and the relationship between seedling growth parameters was examined. Significance between control and treatment was compared at 0.05 probability levels.

RESULTS

Morphological changes: The higher concentration (60 and 80%) of both, fresh leaves as well as leaf litter leachate extract significantly reduced the germination percentage, root length, shoot length and dry biomass and chlorophyll content of *Salvadora* seedlings. At 80% concentration of leaf litter leachate extract, the germination percentage was reduced by 52% and at the same concentration of the fresh leaves extract, it was reduced by 47% (Fig. 1a). The reduced significant

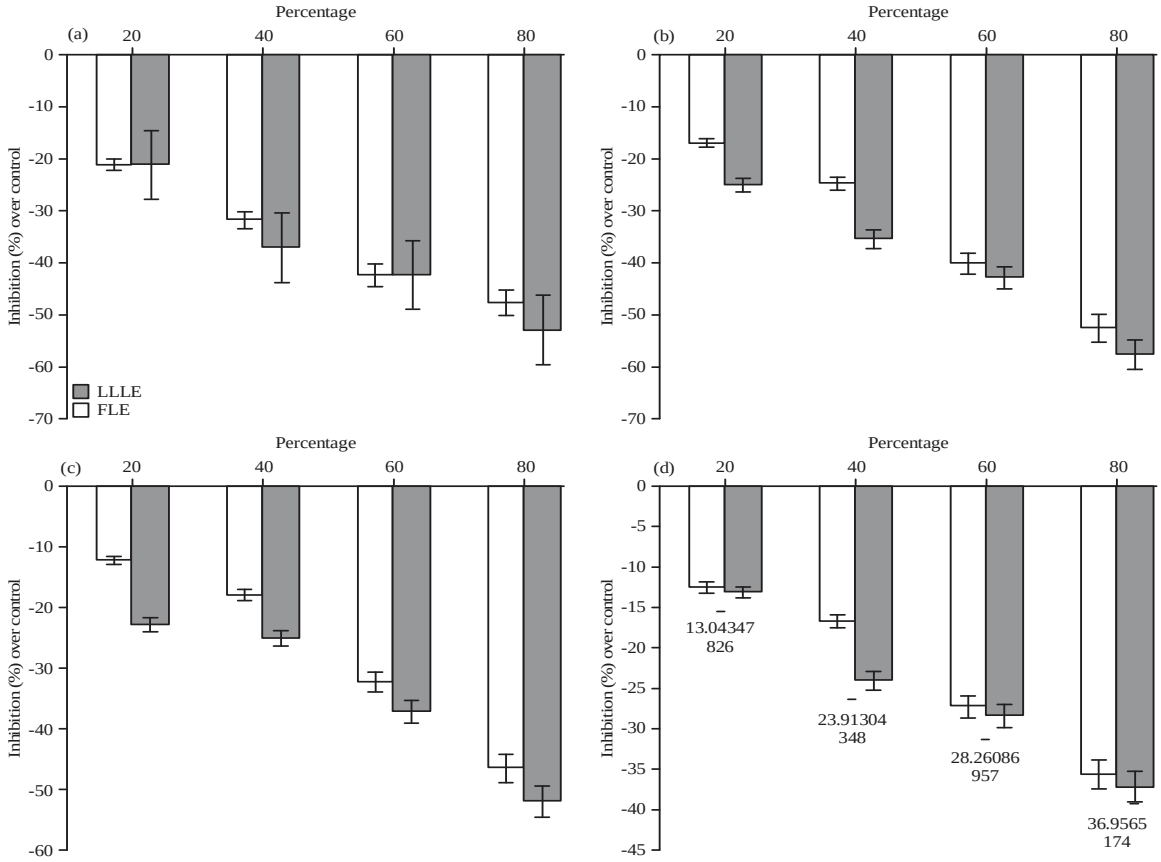

Fig. 1(a-d): Effects of fresh leaf extract (FLE) and leaf litter leachate (LLLE) of *C. odorata* on (a) Germination (%), (b) Seedling growth (shoot length), (c) Root length and (d) Dry matter of *Salvadora persica*

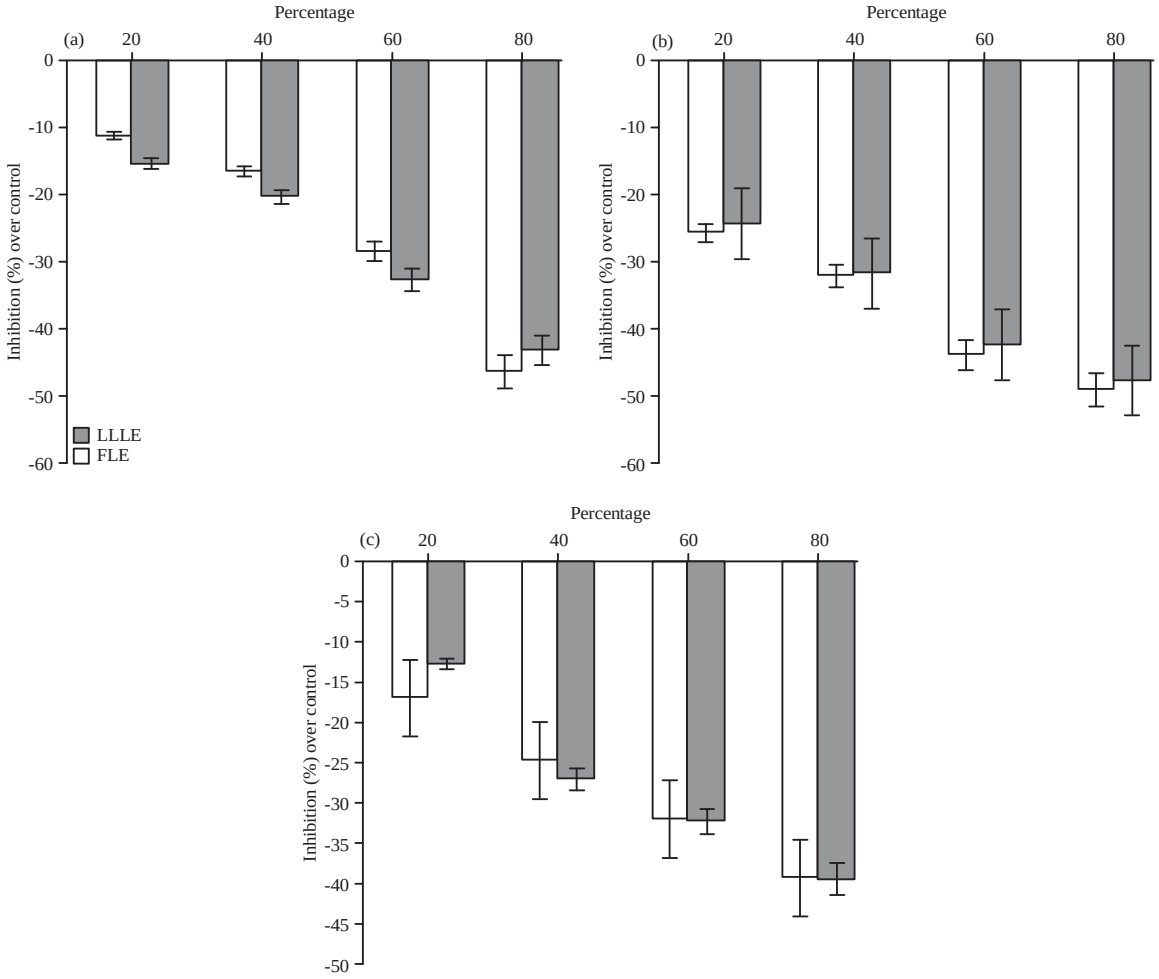

Fig. 2(a-c): Effects of fresh leaf extract (FLE) and leaf litter leachate (LLLE) extract of *C. odorata* on chlorophyll content, (a) Chl a-, (b) Chl b- and (c) Total Chlorophyll of *Salvadora persica*

trend in root and shoot length of *Salvadora* seedling occurred more at 80%. The percentage reduction in shoot length (57.33%) and root length (51.78%) observed at 80% leaf litter leachate extract (Fig. 1b and c). The dry biomass reduction significantly noticed more at 80% of all extract types, but the retardation effect varies with extract type, more in leaf litter leachate extract reduced by 37% followed by fresh leaves extract (Fig. 1d).

Biochemical changes: Both leaf extracts significantly decreased the amount of chlorophyll content. The maximum significant decrease in chlorophyll content was found with leaf litter leachate extract at 80% as compared to control (Fig. 2 a, b and c). The soluble protein content of *Salvadora* was reduced by 50-60% due to the leaf litter leachate extract and fresh leaf extract of *C. odorata* (Fig. 3a). The maximum reduction (32-36%) of total sugar was observed at 80% leachate concentration over control (Fig. 3b). The free proline content in *Salvadora* seedlings increased with increasing extract concentrations. At 80% leachate concentration nearly 26% increase in proline was observed over control (Fig. 3c). The phenol content in *Salvadora* seedling increased with increasing extract concentrations than control (Fig. 3d). The maximum stimulation (37%) of phenol content was observed at 80% leaf litter leachate concentrations (Fig. 3d).

There was a significant reduction ($p<0.05$) between chlorophyll and shoot length at 80%, leaf litter leachate concentration (%). The significant reduction ($p<0.05$) at 80% concentration was also observed in shoot length and phenol (Table 1).

Phytochemical analysis: Mass spectral analysis of bioactive volatile compounds from leaf litter leachate and fresh leaves of *Chromolena odorata* was shown in Table 2. Both the

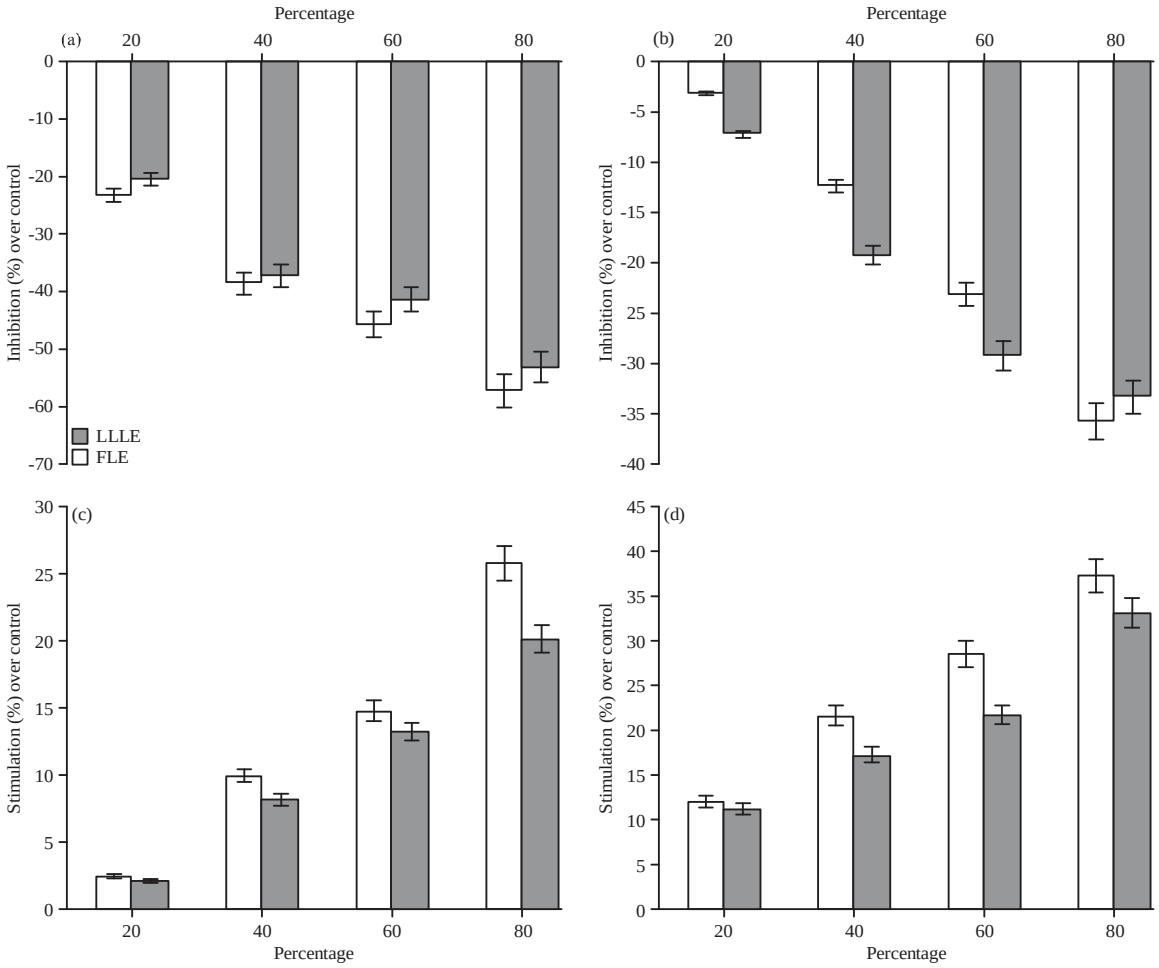

Fig. 3(a-d): Effects of fresh leaf extract (FLE) and leaf litter leachate (LLLE) extract of *C. odorata* on biochemical parameters of *Salvadora persica*, (a) Soluble protein content, (b) Total sugars, (c) Proline and (d) Phenol content

Table 1: Pearson correlation of biochemical parameters of test plants

Leaf litter leachate	Concentration (%)	Parameters level of significance
40	Phenol and chlorophyll	p<0.01
60	Phenol and proline	p<0.01
80	Chlorophyll and shoot length	p<0.05
	Shoot length and dry matter	p<0.01
	Phenol and soluble sugars	p<0.01
	Shoot length and phenol	p<0.05

extracts were rich in bioactive volatile compounds. Among these volatile compounds, some represented the class of monoterpenes, alkenes and alcohols. The major fatty acids found in leaf litter leachate extract were n-hexadecanoic acid, Cyclopentadecanone, Oleic acid, Octadecanal and 1,2-benzenedicarboxylic acid. Whereas, in leaf extract, the major fatty acids were n-hexadecanoic acid, Oleic acid, Octanoic acid, 2-Heptenal and 1,2-benzenedicarboxylic acid.

DISCUSSION

In the present investigation fresh extract and leaf litter leachate extracts of various concentrations of *C. odorata* had varying degrees of inhibition on the germination and growth of *Salvadora* seeds, reflects the allelopathic potential of the plant. According to Tawaha and Turk[26], lowering in germination rate due to allelo-chemical stress may be because of inhibition of water uptake. Cell division and elongation, which are growth prerequisite are known to be inhibited by allelo-chemical. Oudhia[27] in his study reported that the inhibition of seed germination was found to be concentration-dependent. Gulzar and Siddiqui[28] also reported that the aqueous extracts of various concentrations of leaf, fruit and flower of *C. procera* had varying degrees of inhibition on the germination and growth of mustard seeds, reflecting the allelo-pathic potential of the plant.

Table 2: Compounds detected in GC-MS/MS analysis in the leaf litter leachate (LLLE) and fresh leaf extract (FLE) of *Chromolena odorata*

Mol. wt.	Name of compound	LLLE area of peak (%)	FLE area of peak (%)
256	n-Hexadecanoic acid	22.05	18.53
240	Cyclopentadecanone	10.04	0.00
282	Oleic acid	7.20	5.37
268	Octadecanal	6.14	2.10
390	1,2-benzenedicarboxylic acid	5.10	4.14
112	2-Heptenal	3.42	4.47
154	2-Decenal	3.16	3.08
144	Octanoic acid	3.10	4.57
228	Tetradecanoic acid	2.98	0.00
102	2-Hexanol	2.94	0.00
142	Nonanal	2.70	4.18
618	Tetratetracontane	2.49	2.80
338	Tetracosane	2.43	3.25
270	Hexadecanoic acid	2.40	2.48
254	Heptadecane	2.31	0.00
296	Heneicosane	2.27	0.00
268	Oxirane	1.70	0.00
492	Pentatriacontane	1.35	3.60
126	2-octenal	1.31	4.38
282	2-nonadecanone	1.20	2.34
152	2,4-decadienal	0.96	2.63
210	1-pentadecene	0.25	0.96
249	9,12-Octadecadienoic acid	0.00	2.12
182	1-tridecene	0.00	3.76
324	4,8,12,16-tetramethylheptadecan-4-olide	0.00	3.01
158	Dec-2-en-1-ol	0.00	3.45
168	2-undecenal	0.00	3.20
256	Eicosanoic acid	0.00	2.12
100	Hexanal	0.00	2.29
252	1-Octadecene	0.00	0.77
	Total	87.50	89.60

The decrease in the chlorophyll concentration of *Salvadora* in all concentration of both extracts further reflected the reduction or decrease in the total soluble sugar. Reduction in chlorophyll content was previously reported as a result of allelo-chemical stress by several workers[29-31] which could be attributed to the inhibition of chlorophyll biosynthesis and/or the stimulation of chlorophyll[32]. Desai and Gaikwad[33] reported the reduction in chlorophyll content of rice cultivars due to the allelo-chemicals present in the leachate of mangrove *Excoecaria agallocha*. In this comparative study, though all both extract showed significant allelo-pathic potential, the degree of inhibition seemed to be highest in the case of the leaf litter leachate extract of *C. odorata*. According to Tripathi *et al.*[34] the reduction in photosynthetic pigments is due to adverse effect of allelo-chemical stress. They further stated that the reduction in chlorophyll contents observed in all the concentrations might be due to degradation of chlorophyll pigments or other phytochemicals present in leaf leachates. In view Pawar and Chavan[35], reduction in chlorophylls may decrease the photosynthesis and thereby substantially decrease further metabolites like total sugars, proteins and soluble amino acids. The reduction in chlorophyll content by leachates might be due to (i) Degradation of chlorophyll pigments or reduction in their synthesis and (ii) Action of flavonoids, terpenoids or other phytochemicals present in leaf litter leachate[36]. Increase in phenol contents was also responsible for reducing the seedling growth. The increase in phenol contents was correlated with the reduction in seed germination and seedling growth of *Salvadora*. The accumulation of proline content during the allelopathic effect in sorghum was reported by Pawar and Chavan[35]. According to them, the proline accumulation under stress conditions was mainly due to increased synthesis from glutamate. Increased proteolysis in germinating seeds can lead to an increase in free proline content along with other amino acids.

Both the extracts showed some allelo-chemical compounds. Xian *et al.*[37] reported several fatty acids with allelopathic activity. The myristic acid and palmitic acid inhibited the plant growth[38]. Geethambigai and Prabhakaran[39] studied the allelopathic potential of *Cyperus* and *Cynodon* on germination and growth of some rice cultivars and found that the weed *C. dactylon* contains beta-sitosterol, beta-carotene, vitamin C, palmitic acid and triterpenoids. According to Singh *et al.*[40] the most common allelo-chemicals include cinnamic and benzoic acids, flavonoids and terpenes. According to Einhellig[41], these compounds were phytotoxic. In view of John and Sarada[42], phenolic allelo-chemicals inhibited the plant root elongation, cell division, change the cell ultra-structure and subsequently interfered with normal growth and development of the whole plant. Desai and Gaikwad[33] studied the allelopathic effect of a mangrove *Excoecaria agallocha* on rice, they also reported some growth inhibitory allelo-chemicals in their study. Hence, if the plant present nearby agriculture land, they may disturb the stand establishment of cash crops. Thus, there is a need to take serious call on the presence of this plants near the crop filed. Further research can explore the effect of allelochemicals present in *C. odorata* as well as the allelopathic mechanism on other crop plants, through which this phytotoxic plant distracts the neighboring plants.

CONCLUSION

The extract of the weed *C. odorata* inhibited the germination and seedling growth of *Salvadora* due to its phytotoxic effects. The allelo-chemicals present in the weed extracts might be jointly synergistic to seeds germination and

seedling growth of finger millet. Similarly, the phytotoxicity of *C. odorata* in this study might be due to the interactions of various groups of fatty acids and phenols. Hence, if present in the field, this weed can disturb the stand establishment of neighbouring plants. There is a need to take a serious notice of the presence of this weed in the crop fields and nearby places. Experiments to investigate its allelopathic behaviour on other crop plants and to better understand the role of this weed plant in structuring cropping in coastal areas.

SIGNIFICANCE STATEMENT

This study elucidate that the aqueous leaf extracts of the siam weed have a wide range of activities and containing allelopathic compounds with strong potential, which may play important role in weed control and could be used as an alternative of chemical compounds.

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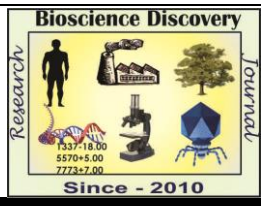

Effect of *Chromolena odorata* leaf extract and leaf leachate on nutrient uptake potential of *Crotalaria verrucosa* L. and *Crotalaria retusa* L.

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Abstract

Allelopathy is biological phenomenon in which biochemical or phytochemicals produced by one organism affects on growth, development and reproduction of other organisms. Such toxicity developing organism is also called as invasive species. *Chromolena odorata* (L.) R. M. King & H. Rob is one of the invasive species from family Asteraceae were selected for study their allelopathic effect on nutritional uptake potential (N, P, K, Ca, Mg, S, Zn, Fe, Mn and …) of *Crotalaria verrucosa* L. and *Crotalaria retusa* L. from this study it is clear that leaf leachate and leaf extract of this invasive weed highly influence micro and macro nutrient contents of studied plant species and directly or indirectly this species inhibit growth and development of other plants. Therefore this plant *Chromolena odorata* can be used to prepare weedicide or herbicide.

INTRODUCTION

Allelopathy refers to the beneficial or harmful effects of one plant on another plant that may be weed species. Allelochemicals are phytochemicals produced by plants that may be released by plant by process like leaching, root extraction, volatilization, residue decomposition and by many other experiment or processes (Patil, 2011).

All living organisms require a continuous supply of large number of substances from outside to complete their life cycle. This supply is called as nutrition. The essential nutrients required by higher plants are exclusively of inorganic nature. A plant for normal optimal growth requires sixteen different elements. Green plants have comparatively simple nutrient requirements and that these are classified as macronutrients (N, P, K, Ca, Mg, S, and Na) and micronutrients (Fe, Mn, Cu, Zn, Mo, B and Cl). Macronutrients are found and needed in plants in relatively higher amounts than micronutrients. But both are essential for almost all metabolic processes.

Allelopathy is biological phenomenon in which biochemicals or phytochemicals produced by one organism affects on germination, growth, development and reproduction of other organisms (Molisch, 1937; Madane and Patil, 2017). Such toxicity developing organism is also called as invasive species. *Chromolena odorata* (L.) R. M. King & H. Rob is one of the invasive species from family Asteraceae were selected and studied in current research for their allelopathic effect on nutritional uptake potential of *Crotalaria verrucosa L. and Crotalaria retusa L.*. Results have been depicted in table 1 and Fig. 1-10.

MATERIALS AND METHODS

Various inorganic constituents like Na^{+}, K^{+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+} were estimated from the *Crotalaria verrucosa*L. and *Crotalaria retusa* L. under allelopathic treatment of *Chromolena odorata* leaf extract and leaf leachates treatments. Oven dried plant material was powdered and 0.5 g of sample was acid digested following the standard method of Toth *et al.* (1948).

Plant material samples were taken in a 150 ml clean borosil beaker and to that 10 ml concentrated HNO_3 were added. It was covered with watch glass and kept for an hour till the primary reactions subsided. It was then heated on hot plate till all the material was completely dissolved. It was allowed to cool to room temperature and then 10 ml of Perchloric acid (60%) were added to it and mixed thoroughly. It was then heated strongly on the hot plate until the solution became colourless and reduced to about 2-3 ml. While heating, the solution was not allowed to dry. After cooling, it was transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day it was filtered through Whatman No. 44 filter paper. The filtrate was stored properly and used for inorganic constituents analysis which was estimated by using flame photometer and Atomic absorption spectrophotometer (Perkin-Elmer, 3030 A) as prescribed by AOAC (1995) and Sangle (2015).

RESULTS AND DISCUSSION

Mineral uptake potential in any plant is always influenced by many edaphic factors such as soil moisture, soil pH and soil microflora. Leaf litter also shows effect on composition of nutrient availability in soil. But sometime it may adversely affects due to allelochemicals released by these litter (Pawar and Chavan, 2007). Many plant species release allelochemicals that (positive or negative) influence on germination, growth and development of other plants (Kadioglue *et al*., 2005 & Madane and Patil, 2017). Here an attempt have been made to study effect of allelopathic compounds from *Chromolenaodorata* on mineral uptake potential in *Crotalaria verrucosa* L. *and Crotalaria retusa*L. plants. The influence of leaf leachates and leaf extract of *Chromolena odorata* was investigated on *C. verrucosa* L. *and C. retusa*. Results have been depicted in table 1 and Fig. 1-10.

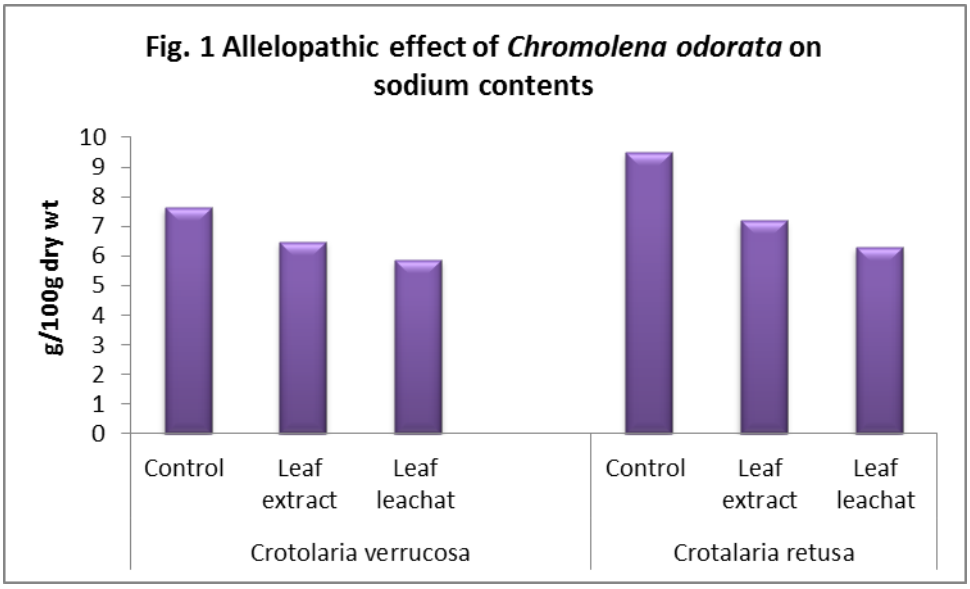
Table 1. Study the effect of leaf leachate and aqueous leaf extract of *Chromolaenaodorata*on mineral uptake potential of *Crotolariaverrucosa and Crotalaria retusa*

Plants under treatment →	*Crotolariaverrucosa*				*Crotalaria retusa*		
Inorganic constituents ↓	Control	Leaf extract	Leaf leachat		Control	Leaf extract	Leaf leachat
Sodium %	7.65	6.47	5.88		9.5	7.2	6.3
Potassium %	6.9	5.4	4.5		7.12	6.37	4.45
Nirogen %	3.24	2.16	1.97		3.68	2.84	2.41
Phosphrous %	0.28	0.21	0.19		0.53	0.32	0.19
Calcium %	8.5	6.2	5.9		9.89	9.05	7.36
Magnesium %	0.27	0.23	0.18		0.94	0.89	0.81
Sulphur %	0.38	0.27	0.22		0.28	0.19	0.13
Ferrous PPM	547.53	490.68	430.63		879.32	764.26	662.84
Mangenese PPM	116.99	102.65	95.62		62.1	59.32	54.68
Copper PPM	46.43	38.28	20.76		183.16	142.42	135.07
Zinc PPM	6.73	6.16	5.08		24.74	15.22	14.47
SD	162.918	146.108	128.648		261.653	227.140	206.635
SEM	49.122	44.053	38.789		78.891	68.485	62.303
P value		0.907	0.803			0.882	0.849

Sodium contents:-

It is evident from result shown in Fig. 1 that due to allelochemicals there is decline in sodium contents were observed in both species *Crotolaria verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining sodium content up to 15.4% and due to leaf leachate 23.1% as compare to control (7.65gm/100gm dry wt.).In *Crotolaria retusa* of leaf extract responsible for declining sodium content up to 24.21% and leaf leachate responsible to decline 33.68% as compare to control (9.5gm/100gm dry wt.). Sodium is considered as accessory element for glycophytes except for few saline angiosperms. Sodium inspires growth at scarcity of potassium supply. It stimulates growth through enhanced cell expansion. It is also required in maintaining membrane integrity (Brownell, 1979).

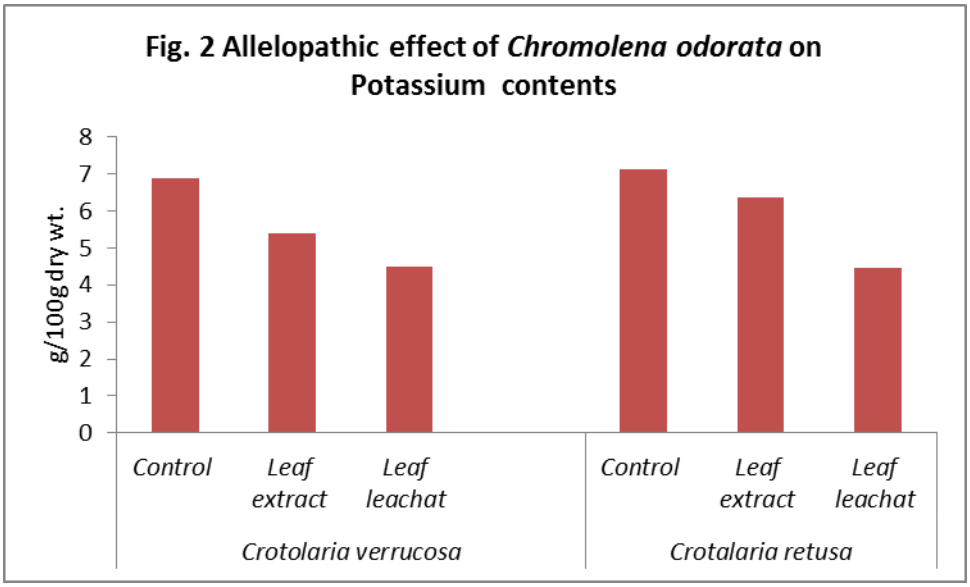
Under sodium deficiency the plants exhibit chlorosis and necrosis, or even fail to form flower. Many C_3 species also benefit from exposure to low levels of sodium ions. According to Chirputkar (1969) adequate level of sodium for glycophytes is 0.6 to 1.4% dry wt. The range of sodium in glycophytes as given by Gauch (1972) is 0.1 to 1.4% dry wt. In crop plants like sugarcane, 0.11% sodium content was recorded by Nimbalkar (1973) and in finger millet it was 0.09% (Chavan, 1980).



Potassium contents

It is evident from result shown in Fig. 2 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in potassium contents were observed in both species *Crotolaria verrucosa* and *C. retusa*. *In C. verrucosa* treatment of leaf extract responsible for declining potassium content up to 21.7% and due to leaf leachate 34.8% decline observed as compare to control (6.9 g/100g dry wt.). In *Crotolaria retusa* of leaf extract responsible for declining potassium content up to 10.53% and due to leaf leachate 37.50% as compare to control (7.12g/100g dry wt.). Plants require 1% potassium for their optimal growth (Epstein, 1972). Potassium is indispensable for plant growth. Potassium is involved in wide range of metabolic activities such as carbohydrate metabolism, glycolysis, phosphorylation and adenine biosynthesis in plants. It also plays a significant role in plant growth and developmental processes such as photosynthesis (Peoples and Koch, 1979), translocation of proteins and carbohydrates (Marschner, 1997), stability of ribosomes, protein synthesis, nitrogen turnover, activation of enzymes, stomatal movement, nyctinastic and seismonastic movements, cell extension, etc. (Suelter 1970, Humble and Raschke 1971, Shankar *et al*., 2013)

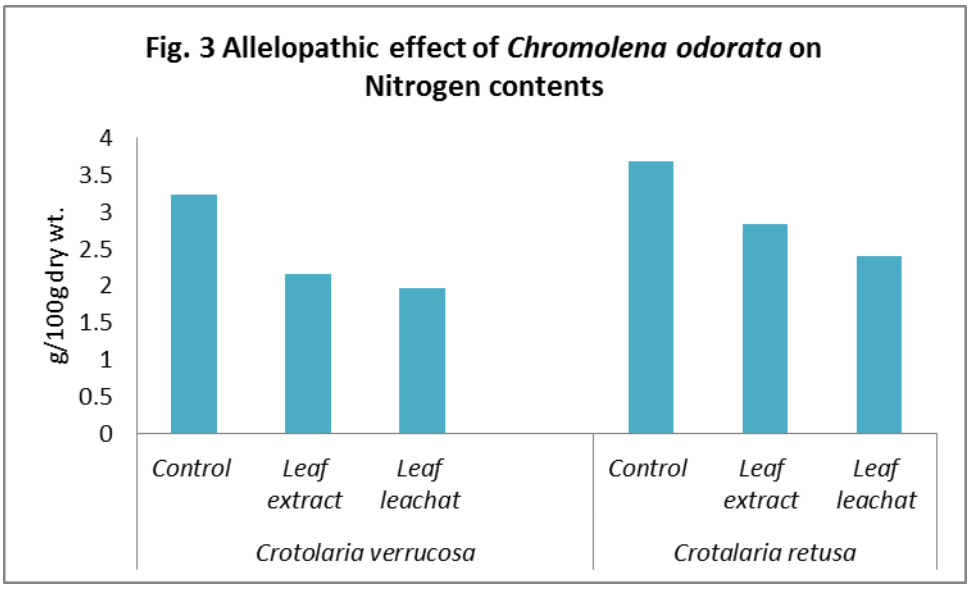
As potassium is highly mobile element (Mengel and Krikby, 1982), in plants its deficiency is usually observed in older leaves, which become chlorotic and develop necrotic patches. Potassium deficiency is usually noted in mature leaves which become chlorotic and develop dark necrotic lesions. Potassium deficiency causes reduction in nitrate reductase activity, disturbance of protein metabolism and accumulation of amino acids and soluble organic nitrogenous compounds.



Nitrogen contents

It is depicted from result shown in Fig. 3 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in nitrogen contents were observed in both *C. verrucosa* and *C. retusa*.*In Crotolaria verrucosa* treatment of leaf extract responsible for declining nitrogen content up to 33.3% and that due to leaf leachate 39.2% as compare to control(3.24 g/100g dry wt.).Similarly in *C. retusa* of leaf extract responsible for declining nitrogen content up to 22.83% and that due to leaf leachate 34.51% as compare to control (3.68g/100g dry wt.) one. Nitrogen is the most essential macronutrient required in life cycle of plants. Nitrogen is a fundamental constituent of amino acids, proteins and nucleic acids, biochemistry of enzymes, pigments, secondary metabolites and polyamines (Maathuis, 2009). It involve in the biosynthesis of several vitamins such as biotin, thiamine, niacin and riboflavin.

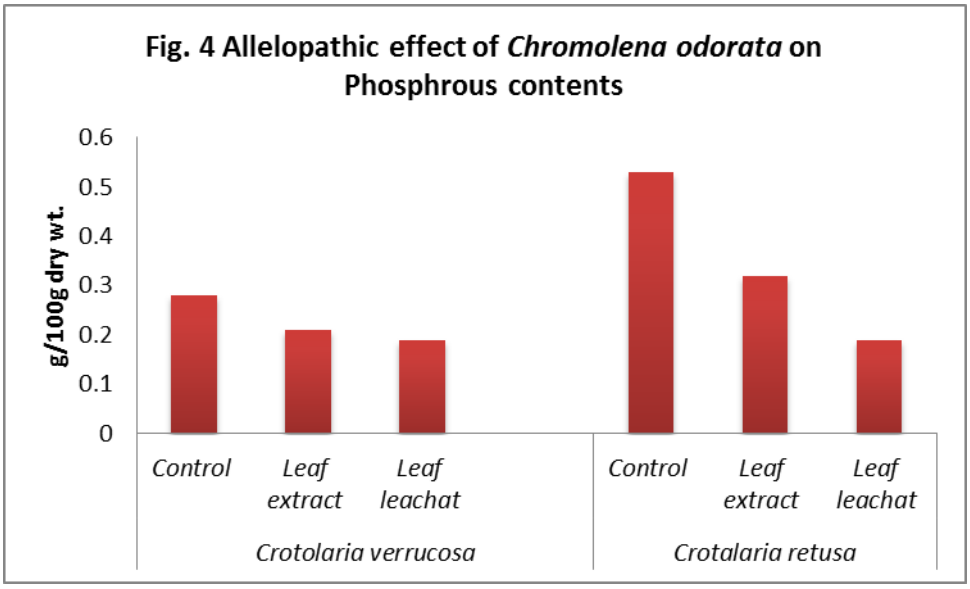
The critical amount of nitrogen 1to 2% requires by plants and maximum amount ranges in between 4 to 6% (Parsa and Bagheri, 2008). Many researchers reported and supported to Marschner's (1986) suggestion as the requirement of nitrogen for optimal growth of plants ranges in between 2 to 5% of the dry weight (Pawar 2004, Zhao *et al.* 2008, Shen *et al.* 2008 Rasmussen 2008, Tambe 2009, Desai, 2010 and Sonar, 2014). N deficiency seriously affects the growth, development and yield in the crop plants.



Phosphrous contents

It is depicted from result shown in Fig. 4 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in phosphorus contents were observed in both *C. verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining phosphorus content up to 25.0% and that due to leaf leachate 23.1 % as compare to control (0.28 g/100g dry wt.).Similarly in *C. retusa* of leaf extract responsible for declining phosphorus content up to 39.62% and that due to leaf leachate 64.15% decline as compare to control (0.53g/100g dry wt.) one.

Phosphorus is one of the most essential components of large number of metabolites it has vital functioning in various life processes. It is absorbed in the form of dihyrogen phosphate ion from the soil solution. It act as backbone in DNA and RNA molecules, act as structural component of ATP molecule. In plant body phosphorous in its inorganic state plays a very important role in regulation of various pathways like photosynthesis and carbohydrate metabolism (Marschner, 1986) and optimal growth of plants the requirement of P is in the range of 0.3 to 0.5% of plant's dry weight.

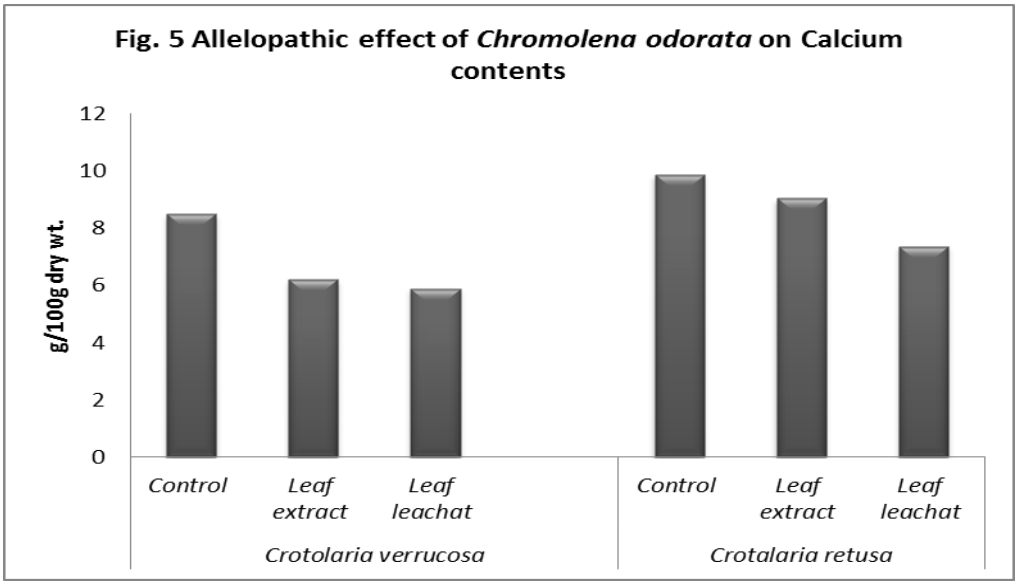


Calcium content

It is clear from result shown in Fig. 5 that due to leaf extract and leaf leachate treatment of *Chromolena odorata*, there is decline in calcium contents were observed in both *C. verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining calcium content up to 27.1% and that due to leaf leachate 30.6 % as compare to control(8.5 g/100g dry wt.). Similarly in *C. retusa* of leaf extract responsible for declining calcium content up to 8.49% and that due to leaf leachate 25.58% decline as compare to control (9.89g/100g dry wt.). Calcium has many roles in plants and is required in differing amount depending on the process in which it is involved, from minute amount in regulating some aspects of cytosolic metabolism to macro amount in cell wall structure. The importance of calcium in functioning of membrane and maintenance of cell integrity as well as synthesis of pectin in middle lamellae of cell wall is very well documented.

Calcium has long been known to be essential for structural and functional integrity of plant membrane (Epstein1972 and Miller et al., 1992),. According to Clark (1984), the activities of many enzymes have been either stimulated or inhibited by calcium. Calcium is also a non- toxic mineral nutrient, even in high concentration and is very effective in detoxifying high concentrations of other mineral elements in plants. The major role carried out by calcium in plants is to bind with proteins, nucleic acids and lipids to affect cell adhesion, membrane chromatin organization and enzyme conformation (Clarkson and Hanson, 1980).

Under calcium deficiency, activities of glutamine synthase and nitrate reductase are decreased, while that of GDH is increased, root tips appear slimy, death of terminal buds takes place, young leaves develop rugged edges and die back of leaves takes place.

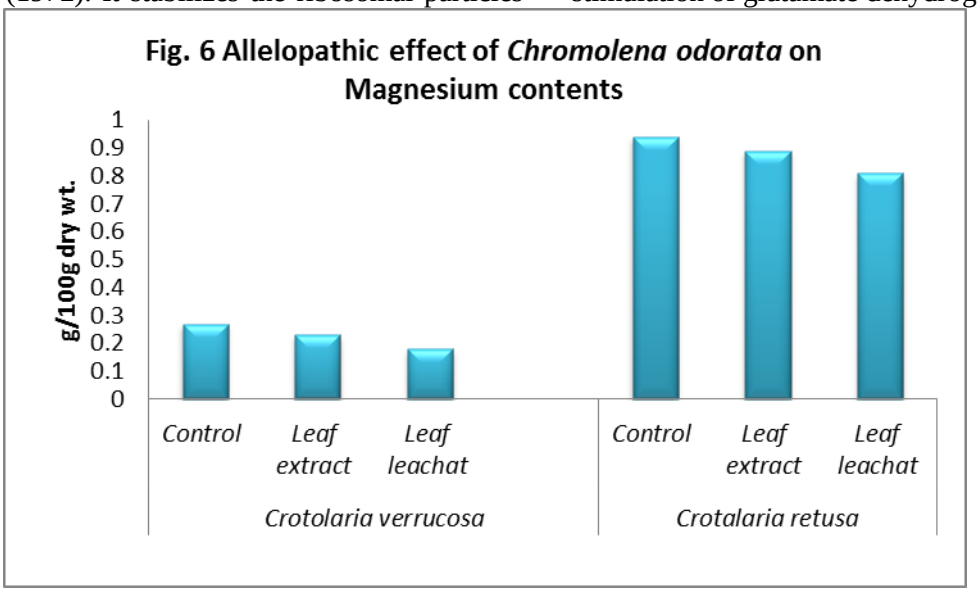


Magnesium content

From result shown in Fig. 6 it is evident that due to allelopathic treatment of *Chromolena odorata*, there is decline in magnesium uptake were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining magnesium uptake up to 14.8% and that due to leaf leachate 33.3% as compare to control (0.27 g/100g dry wt.). Similarly in *C. retusa* of leaf extract responsible for declining magnesium uptake up to 5.32% and that due to leaf leachate 13.83% decline as compare to (0.94g/100g dry wt.) control one. In the plants, 2% Mg on dry weight basis has been regarded as critical value by Epstein (1972). It stabilizes the ribosomal particles in the configuration necessary for protein synthesis (Mengel and Kirkby, 1982).

Magnesium is a small, mobile and strongly electropositive divalent cation in the plants, found both in bound as well as free form (Gilbert, 1957). Most well known role of Mg is its contribution to the center of the chlorophyll molecule. It is a part of ring structure of chlorophyll molecule, the photosynthetic pigment in chloroplast. It is a cofactor of several enzymatic reactions involved in organic acid synthesis.

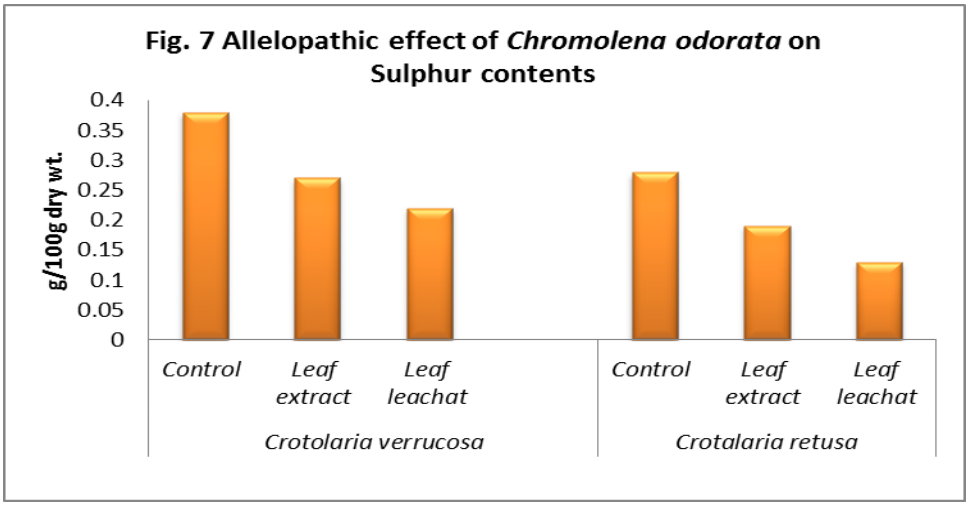
Mg deficiency leads to mottled and chlorotic leaves, interveinaly following or necrosis of older leaves, reduction in activities of nitrate reductase and stimulation of glutamate dehydrogenase.



Sulphur contents

According to result as shown in Fig. 7 it can be said that allelopathic treatment of *Chromolena odorata* responsible for decline in sulphur uptake. Which were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining sulphur uptake up to 28.9% and that due to leaf leachate 42.1% as compare to control(0.38 g/100g dry wt.). Similarly in *C. retusa*of leaf extract responsible for declining Sulphur uptake up to 32.14% and that due to leaf leachate 53.57% decline as compare to control (0.28g/100g dry wt.). Sulphur act as secondary essential plant nutrient is usually required by crops in amounts comparable to phosphorus. The optimum value of sulphur in plant species is in the range 0.08 to 1.56 % (Munson, 1998). Mengel and Kirkby (1978) reported that total sulphur requirement of different crops depends on the plant biomass production and varies among the crop species and crops with a high production of organic material have a high demand for sulphur. Sulphur being a part of amino acids as cysteine and methionine. It is involved in the formation of chlorophyll (Tandon 1991, higher levels of structural organization in proteins. Rouhier*et al.* (2006) reported that sulphur present in tripeptide glutathione which helpful in oxidative stress tolerance. The lower sulphur content of proteins influences nutritional quality considerably.

Deficiency of sulphur results in the inhibition of protein synthesis. Griffiths *et al.* (1995) reported that decline in the enzyme activity can cause a reduction in S level in senescent leaves.

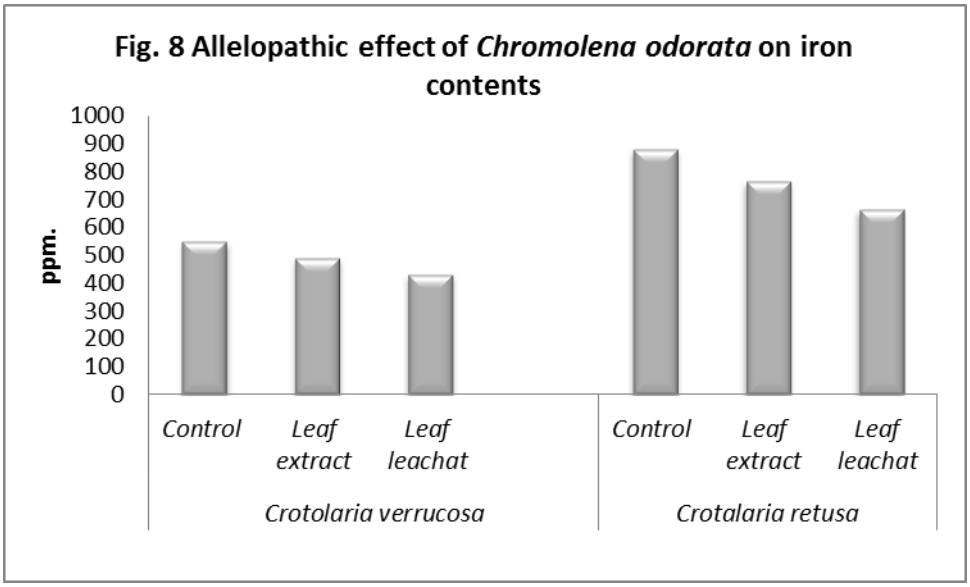


Iron contents

In current research as result shown in Fig. 8 indicates that due to allelopathic treatment of *Chromolena odorata*, there is decline in iron accumulation were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining iron accumulation up to 89.6% and that due to leaf leachate 78.6% as compare to control(547.53 ppm). Similarly in *C. retusa*of leaf extract responsible for declining iron accumulation up to 13.09% and that due to leaf leachate 24.62% decline as compare to control (879.32 ppm) one.

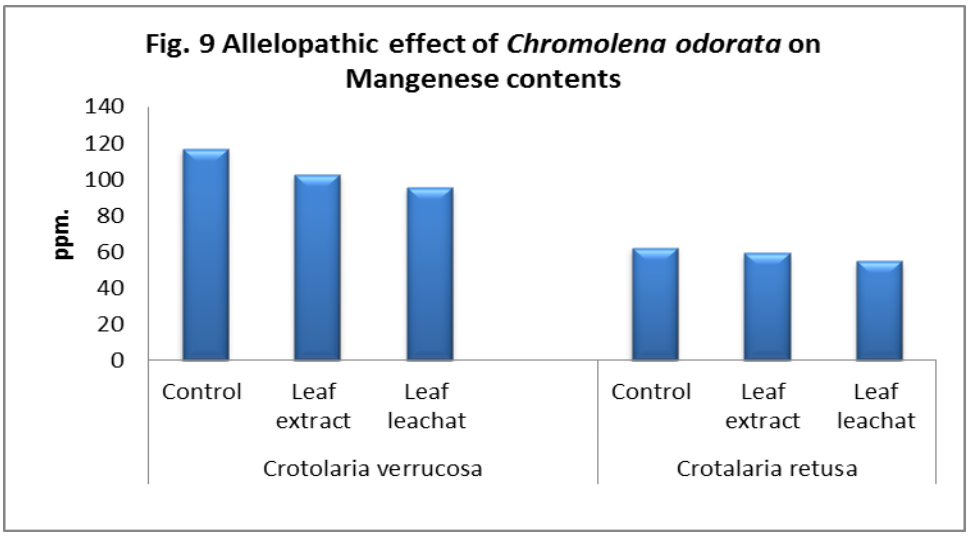
Iron is an immobile element in living cells. It is absorbed by plant roots as Fe^{2+} or as Fe chelate. Fe chelates are soluble and therefore available to roots. The adequate value of iron for optimal growth of plants is 100 ppm (0.01%) (Stout, 1961 and Epstein, 1972). The cytochromes and ferredoxins are the examples of hemeprotein and iron sulphur protein respectively, which act as electron transmitters in a number of basic metabolic processes in chloroplasts and mitochondria. According to Machold and Stephan (1969), iron has role in the synthesis of common precursors of chlorophyll. It is involved in oxidation, reduction reactions, ferredoxin formation and chlorophyll synthesis (Spillar and Teny, 1980). Fe is stored in stroma of chloroplast as phytoferritin, which can store about 5000 atoms of Fe^{3+} (Marschner, 1986).

Iron deficiency causes reduction of nitrate reductase activity and chlorosis (Perur*et al.*, 1961). However, increase in leaf Fe content may cause severe cellular damage.



Manganese contents

It can be depicated from result shown in Fig. 9 that due to allelopathic treatment of *Chromolena odorata*, there is decline in manganese content were observed in both *C. verrucosa* and *C. retusa*. *In Crotolaria verrucosa* treatment of leaf extract responsible for declining manganese content up to 12.3% and that due to leaf leachate 18.3% as compare to control(116.99ppm). Similarly in *C. retusa* of leaf extract responsible for declining manganese content up to 4.48% and that due to leaf leachate 11.95% decline as compare to control (62.1 ppm) one. Manganese plays an important role in the chloroplast membrane system as well as in photolysis of water and O_2 evolution during photosynthesis. Manganese is associated with photosynthesis, respiration, oxidation of carbohydrates and IAA and activation of enzymes of nitrogen metabolism. Enzymes of Kreb's cycle require manganese as a cofactor. According to Marschner (1986), it is directly involved as a component of the biotin enzyme in the biosynthesis of fatty acids.. Manganese is involved in shikimic acid pathway and enhances the resistance of plants to various diseases. Superoxide dismutase incorporating manganese in mitochondria plays an important role in scavenging of free radical (Jimenez *et al.*, 1998). Dietary recommendations established by the Food and Nutrition Board of the Institute of Medicine (IOM) (2004) suggested that 1.8 to 2.3 mg/day intake of Mn is required daily.

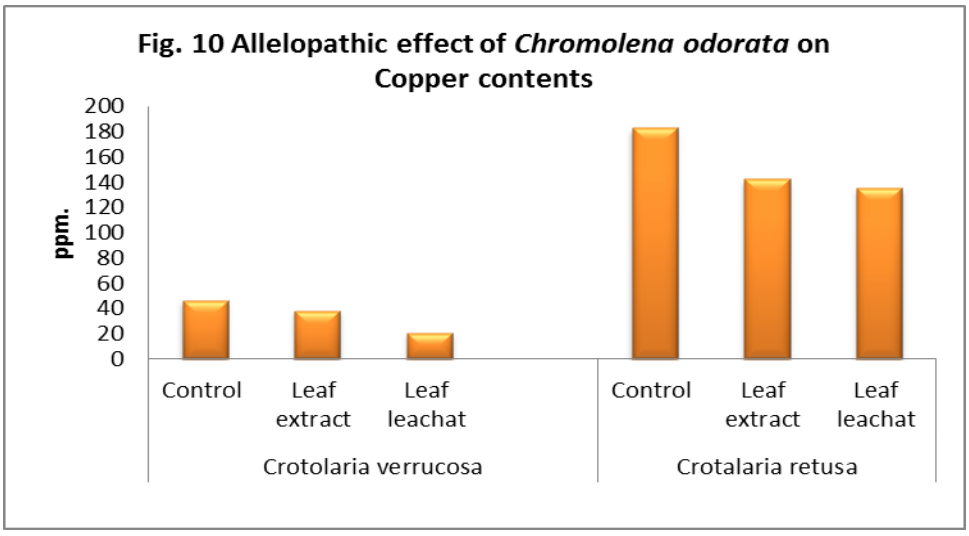


Copper contents

From result shown in Fig. 10 it is evident that due to allelopathic treatment of *Chromolena odorata*, there is decline in copper content were observed in both *C. verrucosa* and *C. retusa. In Crotolaria verrucosa* treatment of leaf extract responsible for declining copper uptake up to 17.6% and that due to leaf leachate 55.3% as compare to control(46.43ppm). Similarly in *C. retusa*of leaf extract responsible for declining copper uptake up to 22.24% and that due to leaf leachate 26.26% decline as compare to control (183.16 ppm).

Copper as a cupric ion is an essential trace element for algae and higher plants (Sommer, 1945; Walker, 1953). Copper provides metabolic control over auxin synthesis (Skoog,1940) and is also involved in protein and carbohydrate metabolism. It plays a vital role in reproductive growth as well as another trace element whose requirement is known in photosynthesis. It also plays an important role in nitrogen metabolism (Hallsworth *et al.,* 1960), being involved in the reduction of nitrate.The critical deficiency level of copper in vegetative parts is generally in the range of 3 to 5 µg g^{-1} (0.0003-0.0005%) dry wt. depending on the plant species, plant organ, developmental stage and nitrogen supply, this range can be larger (Robson and Reuter, 1981).

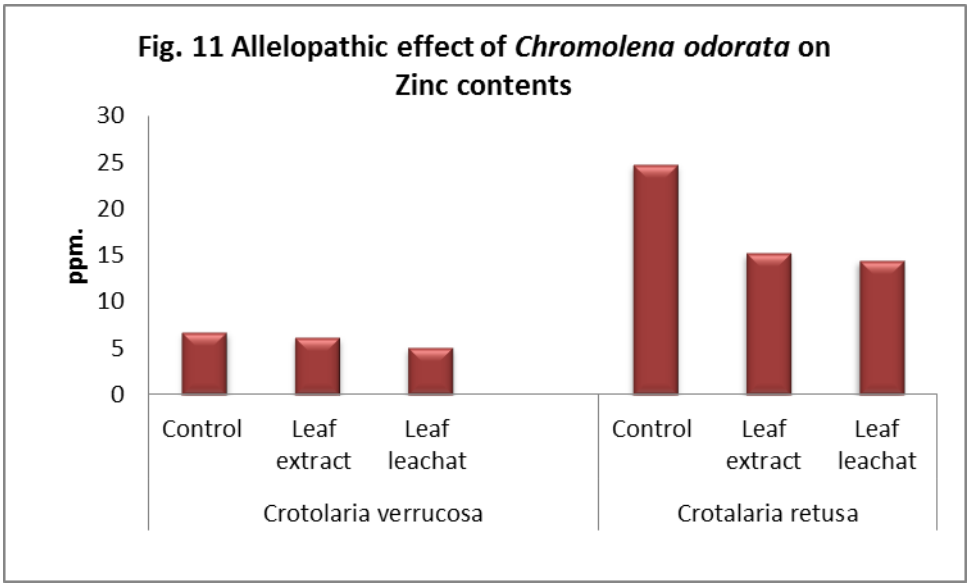
Deficiency of copper induces the activity of ferric reductase enzyme, involved in Fe uptake (Kochian, 2000). According to Mizumo *et al*. (1982), the copper deficient leaves exhibit low soluble carbohydrates than normal leaves during vegetative stage.



Zinc contents

Zinc is essential for carbohydrate metabolism and regulation of consumption of sugars, nitrogen metabolism, protein synthesis, auxin synthesis, particularly IAA synthesis, as well as for sexual fertilization and development of reproductive parts. Many enzymes require zinc for their activity. Zinc acts either as a metal component or as a functional, structural or regulatory cofactor of a large number of enzymes. It is required for chlorophyll biosynthesis. It participates in synthesis of indole acetic acid from its precursor, tryphtophan (Skoog, 1940 and Tsui, 1948). From result shown in Fig. 11 it is clear that due to leaf extract and leachate both treatments of *Chromolena odorata* responsible to decline in zinc content in both *C. verrucosa* and *C. retusa.In Crotolaria verrucosa* treatment of leaf extract responsible for declining zinc content up to 8.5% and that due to leaf leachate 24.5% as compare to control(6.73 ppm). Similarly in *C. retusa*of leaf extract responsible for declining zinc uptake up to 34.48% and that due to leaf leachate 41.51% decline as compare to control (24.74 ppm)). Zn plays a role in membrane stability by regulating the level of oxidizing O_2 species (Pinton *et al.*, 1994).The critical deficiency levels of Zn are below 15–20 mg kg^{-1} dry weight of leaves and critical toxicity levels of zinc in leaves of crop plants are more than 400–500 mg kg^{-1} dry weight basis (Marschner, 1986).

Zinc deficiency is characterized by a reduction in internodal growth resulting into rosette habit of a plant. Zn deficiency in wheat plant decreases NR activity and NO_3 content (Harper and Paulsen, 1969).



It can be concluded from result that *Chromolena odorata* responsible to decline in nutrient uptake potential due to having allelochemicals in it. Leaf leachate of *Chromolena odorata* highly responsible to decline inorganic composition of other species. i. e. it inhibit growth and development of other plants by affecting nutrient uptake potential. Therefore this plant *Chromolena odorata* can be used to prepare weedicide or herbicide.

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In vitro Antioxidant Activities and Antimicrobial Efficacy of Asian Snakewood; *Colubrina asiatica* (L.) Brong.

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ABSTRACT

The present study evaluated antimicrobial efficacy of essential oil and *in vitro* antioxidant activities of the aqueous extract from *Colubrina asiatica* (L.) Brong. as well as the chemical composition of essential oil. In the present investigation, *Colubrina* Water Extract (CWE) was studied for its antioxidant activity and *Colubrina* Essential Oil (CEO) for anti microbial properties. The antioxidant properties of CWE were evaluated using different free radical scavenging assays, such as reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. We found that CWE had powerful antioxidant activity. The different concentrations (50, 100 and 250 g) of CWE showed 39, 66 and 98% inhibition on peroxidation of linoleic acid emulsion, respectively, while 60 g mL^{-1} of ascorbic acid, exhibited only 30% inhibition. Moreover, CWE had effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities at the same concentrations. Those various antioxidant activities were compared to standard antioxidants such as ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), gallic acid and quercetin. In addition, total phenolic compounds in the CWE were determined as Gallic acid equivalent. The *Colubrina* Essential Oil (CEO) content quantified showed presence of 10 compounds in which, dodecamethylcyclohexasiloxane has showed the highest (17%) and dehydro-N-[4,5-methylenedioxy-2-nitrobenzylidene]-tyramine showed the lowest percentage (1.9%). Cubebene, comprised of 14%. The antimicrobial activity of oil was studied on gram negative and gram positive bacterial.

Key words: Essential oil composition, phytochemical screening, *Colubrina asiatica* Brong

INTRODUCTION

Medicinal plants play an important role in human life to combat diseases since time immemorial. The villagers and the tribals in India even today largely depend on the surrounding plants/forests for their day-today needs. Medicinal plants are being focused upon not only as a source of health care but also as a source of income. The Ministry of Environment and Forests, Government of India, reveals that there are over 8000 species of medicinal plants grown in the country. About 70% of these plants are found in the tropical forest; spread across the Western and Eastern Ghats. The Export-Import Bank of India, in its report for the year 1997, puts medicinal plants related trade in India at $.5.5 billion and the same is growing rapidly (Kumar and Janagam, 2011). Free radicals are highly reactive species produced in the body during normal metabolic

functions. These are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. Though Oxygen, is essential to life, but it is the source of the potentially damaging free radicals. Antioxidants counteract these cellular by-products, called free radicals and bind them before they can cause damage (Pandey *et al*., 2005). Fruits and vegetables are major source of dietary antioxidants and their precursors (Block *et al*., 1992). Recently, various phytochemicals and their effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, spices and herbs, have been intensively studied (Ho *et al*., 1994). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) (Sherwin, 1990).

Among the plants with promising biological activities employed by the traditional people, *Colubrina asiatica* (Brong.), is a Rhamnaceae species popularly known as "Asian snake wood" with a glabrous, scandent or sprawling shrubby nature. In India the species is wide spread in littoral scrub forests, tidal forests of Orissa and Ghats of Konkan (Thaman, 1992). In some regions of Northeast and Southeast it is popularly employed in medicinal preparations for the treatment of digestive aid, antiscorbutic (counteracts scurvy), tonic, laxative, a febrifuge, medicinal bath and a vermifuge and skin diseases (Burkill, 1966; Morton, 1981; Austin, 1999). The plant is economically valued for its leaf saponins used in soap substitute, used to wash and whiten textile kilts and garments (Richardson *et al*., 2000).

In this sense, this study aimed the phytochemical investigation and *in vitro* evaluation of antioxidant activities of the aqueous extract and antimicrobial efficacy of essential oil of *Colubrina asiatica.*

MATERIALS AND METHODS

Antioxidant activities

Chemicals: Ammonium thiocyanate and gallic acid were purchased from E. Merck. Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2, 4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trichloroacetic acid (TCA) were purchased from Sigma (Germany).

Plant material and extraction: *Colubrina asiatica* Brong. (whole plant) was collected from coastal area of Goa (coordinates 15°27'7" N 73°50'6" E), in December 2011 and identified by Dr. MayurNandikar, a plant taxonomist of Department of Botany from Shivaji University of Kolhapur. A specimen herbarium deposited in Shivaji University, Kolhapur (NMDESAI 001 SUK). The plant material was initially dried in sunlight and then in oven. The dried sample was chopped into small parts with a blender. For water extraction, 20 g dried leaves of *Colubrina* ground into a fine powder in a mill and was mixed with 400 mL boiling water by magnetic stirrer during 15 min. Then the extract was filtered over Whatman No.1 paper. The filtrate was frozen and lyophilized in a lyophilizator at 5 μm Hg pressure at -50°C (Labconco, Freezone 1L). The extract was placed in a plastic bottle and then stored at -20°C until further use.

Total antioxidant activity determination: The antioxidant activity of CWE was determined according to the thiocyanate method (Mitsuda *et al*., 1996). For stock solution; 20 mg lyophilized *Colubrina* Water Extract (CWE) was dissolved in 20 mL water. Then the solution, which contains

different amount of stock CWE solution or standards samples (50, 100 and 250 μg) in 2.5 mL of 0.04 M potassium phosphate buffer (pH 7.0) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). Each solution was then incubated at 37°C in a glass flask in the dark. At intervals during incubation, each solution was stirred for 3 min, 0.1 μL this incubation solution, 0.1 mL $FeCl_2$ and 0.1 mL thiocyanate were transferred to the test tube, which containing 4.7 mL ethanol solution incubated for 5 min. Finally, the peroxide value was determined by reading the absorbance at 500 nm in a UV-1800 UV-Vis spectrophotometer (Shimadzu). During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe^{2+}-Fe^{3+}. The latter Fe^{3+} ions form complex with SCN-, which has a maximum absorbance at 500 nm. Therefore higher absorbance values indicate higher linoleic acid oxidation. The solutions without added CWE or standards were used as blank samples. Five millilitres linoleic acid emulsion is consisting of 17.5 μg Tween-20, 15.5 μL linoleic acid and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5 mL control composed of 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04 M, pH 7.0). All data about total antioxidant activity is the average of duplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of CWE (Duh *et al*., 1999).

Reducing power: The reducing power of CWE was determined according to the method of Oyaizu (1986). The different doses of CWE (50, 100 and 250 μg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 1000×g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide anion scavenging activity: Measurement of superoxide anion scavenging activity of CWE was based on the method described by Liu *et al*. (1997) with slight modifications (Gulcin *et al*., 2003). Superoxide radicals are generated in phenazine methosulphate (PMS)-nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer(16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and 1 mL sample solution of CWE (100 μg mL^{-1}) were mixed. The reaction was started by adding 1 mL of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured against blank samples. l-ascorbic acid was used as a control. Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control (l-ascorbic acid) and A_1 was the absorbance of CWE or standards (Ye *et al*., 2000).

Free radical scavenging activity: The free radical scavenging activity of CWE was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Shimada *et al*. (1992). Briefly, 0.1 mM solution of DPPH• in ethanol was prepared. Then, 1 mL of this solution was added to 3 mL of CWE solution at different doses (50-250 μg). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH• concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R2: 0.9678).

Absorbance = 104.09×[DPPH•]. The DPPH radical concentration was calculated using the following equation:

$$\text{DPPH} \bullet \text{ scavenging effect } (\%) = 100 - \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of CWE (Oktay *et al*., 2003).

Metal chelating activity: The chelating of ferrous ions by the CWE and standards was estimated by the method of Dinis *et al*. (1994). Briefly, extracts (50-250 μg) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of CWE and standards. The control contains FeCl2 and ferrozine (Ilhami *et al*., 2003).

Scavenging of hydrogen peroxide: The ability of the CWE to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer. Extracts (50-250 μg) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of CWE and standard compounds was calculated using the following equation:

$$\text{Percent scavenged } (H_2O_2) = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of CWE and standards (Ilhami *et al*., 2003).

Determination of total phenolic compounds: Total soluble phenolic compounds in the CWE were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using pyrocatechol as a standard phenolic compound. Briefly, 1 mL of the CWE solution (contains 1000 µg extract) in a volumetric flask diluted with distilled water (46 mL). One milliliter of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, 3 mL of Na2CO3 (2%) was added and then was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the CWE determined as microgram of gallic acid equivalent by using an equation that was obtained from standard pyrocatechol graph (Gulcin *et al*., 2002):

Absorbance = 0.0053×total phenols [gallic acid equivalent (µg)]-0.0059

Extraction of oil and GC MS analysis: Hydrodistillation of the plant material was performed in a clevenger-type apparatus for 210 min. The oil obtained was light yellow, liquid at room temperature with an agreeable odor. After isolation, the Essential Oil (EO) was collected and stored in steeled glass vials in refrigerator at 4-5°C. The samples were analysed by GC-MS (Schimadzu) using capillary column. The GC-MS conditions were as follows; injection volume (1 mL), temperature programme 80-160°C for 5 min at 10°C min^{-1}; 160-235°C for 5 min at 5°C min^{-1} and 235-290°C for 5 min at 50°C min^{-1}; injector temperature (280°C), MS transfer line (290°C), ion source (200°C) spit ratio (1: 10) and mass range at 50-450. Data was analysed by comparing it with SI (standard index) from the NIST library available.

Antimicrobial activities

Preparation of test microorganisms: For the purpose of antimicrobial evaluation ten microorganisms were used. *Pseudomonas aeruginosa* (ATCC 9027, gram-negative), *Escherichia coli* (ATCC 9837, gram-negative), *Staphylococcus aureus* (ATCC 6538, gram-positive) and *Streptococcus pneumoniae* (ATCC 49619, gram-positive) microorganism strains were employed for determination of antimicrobial activity. Microorganism strains were obtained from the stock cultures of Microbiology Laboratory, Department of Microbiology, Shivaji University, Kolhapur.

Antimicrobial activity determination: Agar cultures of the test microorganisms were prepared as described by Mackeen *et al*. (1997). Three to five similar colonies were selected and transferred with loop into 5 mL of tryptone soya broth. The broth cultures were incubated for 24 h at 37°C. For screening, sterile, 6 mm diameter lter paper disc were impregnated with 250 µg of the CEO. Then the paper discs were placed onto Mueller Hinton agar. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was adjusted to 108 colony forming units (1×108 CFU mL^{-1}). The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity the average of triplicate analyses. Netilmicin (30 µg per disc), amoxicillin-clavulanic acid (20-10 µg per disc) were used as reference standards, which as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

Statistical analysis: Experimental results concerning this study were Mean±SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. p values<0.05 were regarded as significant and p values<0.01 very significant.

RESULTS AND DISCUSSION

Antioxidant capacity: Antioxidants are the compounds which helps to delay or inhibit the oxidation of lipids and other molecules through the inhibition of either initiation or propagation of oxidative chain reactions (Jaleel *et al*., 2007). Antioxidants can act as either reducing agents, or by free radical scavengers or singlet oxygen quenchers (Chanwitheesuk *et al*., 2005). Recent studies focused on several antioxidant methods and its modifications to evaluate antioxidant activity and to explain how antioxidants function. Among these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O_{\bullet}^{2-}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of extracts (Duh *et al*., 1999; Amarowicz *et al*., 2000; Chang *et al*., 2002). Total antioxidant activity of CWE was determined by the thiocyanate method. The CWE exhibited effective antioxidant activity at all the studied doses. The effects of different amounts of CWE (from 50-250 µg) on peroxidation of linoleic acid emulsion are shown in Fig. 1. The antioxidant activity of CWE was found concentration dependently. The CWE (50, 100 and 250 µg) showed higher antioxidant activities than that of 100 µg concentration of standard antioxidant ascorbic acid. After incubation times the percentage inhibition of peroxidation in linoleic acid emulsion was 34, 62 and 91%, respectively and greater than that of ascorbic acid (30%).

The reductive capabilities of CWE compared to ascorbic acid was shown in Fig. 2. For the measurements of the reducing power ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of CWE samples using the method of Oyaizu (1986). Like the antioxidant activity, the reducing power of CWE increased concentration dependently. All of the concentrations of CWE showed higher activities than the control in a statistically significant ($p<0.05$) manner. In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. Superoxide anion is an initial free radical and plays an important role in the

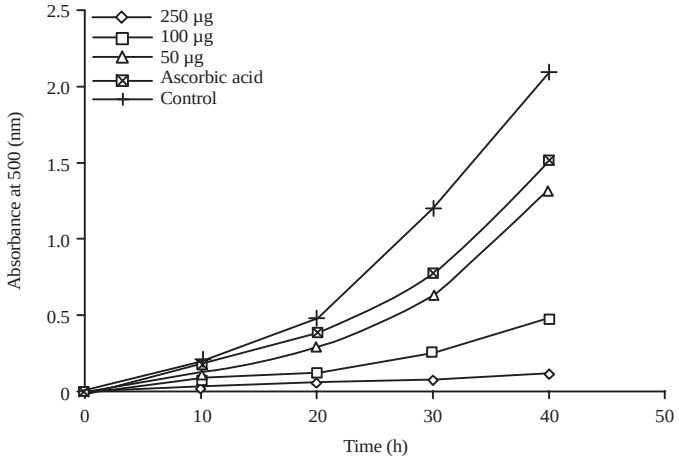

Fig. 1: Antioxidant activity of different doses of CWE and ascorbic acid in the linoleic acid emulsion

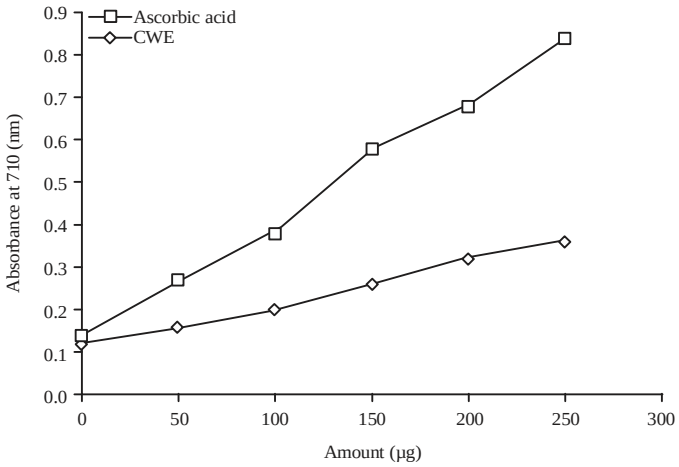

Fig. 2: Reducing power ability of CWE compared with ascorbic acid using spectrophotometric detection of the Fe^{3+}-Fe^{2+} transformation

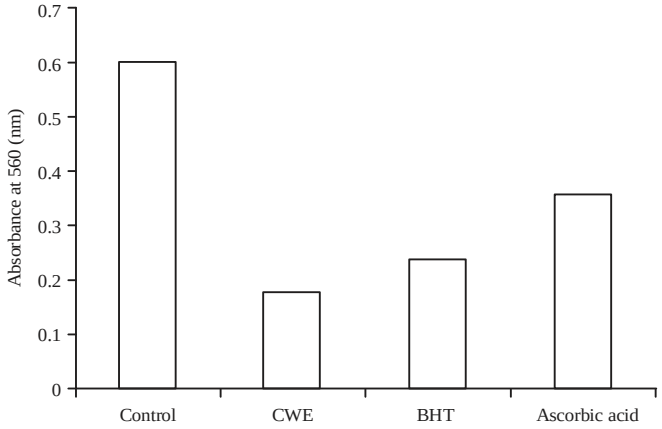

Fig. 3: Superoxide anion radical scavenging activity of 100 g of WEN, BHA, BHT and ascorbic acid by the PMS-NADH-NBT method

formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, or singlet oxygen in living systems (Stief, 2003). It can also react with nitric oxide and from peroxynitrite, which can generate toxic compounds such as hydroxyl radicals and nitric dioxide (Halliwell, 1997). Figure 3 shows the percentage inhibition of superoxide radical generation by 100 µg of CWE and comparison with same doses of BHT and ascorbic acid. The CWE exhibited higher superoxide radical scavenging activity than BHT and ascorbic acid ($p<0.01$). The percentage inhibition of superoxide generation by 100 µg amount of CWE was found as 90% and greater than that of some doses of BHT and ascorbic acid (89, 80 and 61%), respectively. Superoxide radical scavenging activity of those samples followed the order: CWE>BHT >ascorbic acid. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Themodel of scavenging the stable DPPH radical is a widely used method to evaluate

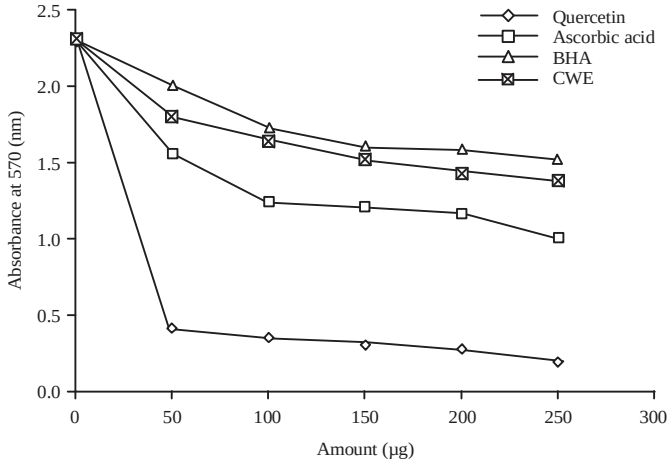

Fig. 4: Free radical scavenging activity of quercetin, ascorbic acid, BHA and CWE on DPPH

antioxidant activities in a relativelyshort time compare to other methods (Soare *et al*., 1997). The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The maximum absorption of a stable DPPH radical in ethanol is at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results inthe scavenging of the radical by hydrogen donation.The DPPH radical scavenging assay depends on the decoloration of the purple coloured methanolic DPPH solution to yellow by the radical scavengers present in the sample extracts (Blois, 1958). The result of DPPH scavenging activity implies that the plant extract may be useful for treating radical related pathological damages (Wang *et al*., 1998). A significant ($p<0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of the CWE and standards was observed (Fig. 4). The CWE and BHA showed almost equal DPPH scavenging activity, however, significantly are lower than that of quercetin. The scavengingeffect of CWE and standards on the DPPH radical decreasedin the order of quercetin>ascorbic acid >CWE>BHA and were 89, 45, 36 and 31% at the concentration of 60 µg mL^{-1},respectively. Uncontrolled generation of ROS can lead to their accumulation causing oxidative stress in the cells (Kunwar and Priyadarsini, 2011). Severe oxidative stress causes cell damage and death (Aruoma, 1998). Superoxide anion radical scavenging activity of 100 µg of CWE, ascorbic acid, BHT and ascorbic acid by the PMS-NADH-NBT method obtained from this study, CWE exhibits free radical scavenging activity as well as a primary antioxidant thatreacts with free radicals, which may hampers the damages caused due to free radical in the human body (Fig. 3). The chelating of ferrous ions by CWE was estimatedwith the method of Dinis *et al*. (1994). In the presence ofchelating agents, Ferrozine can quantitatively form complexes with Fe^{2+} is interrupted and ultimately diminishes the red colour of the complex. The actual mechanism of antioxidant action is chelation of transition metals thus preventing catalysis of hydroperoxidedecoposition and fenton type reactions (Gordon, 1990). Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991). In this assay CWE and standard antioxidant compound interfered with the formation of ferrous and ferrozine complex, suggesting its potent chelating

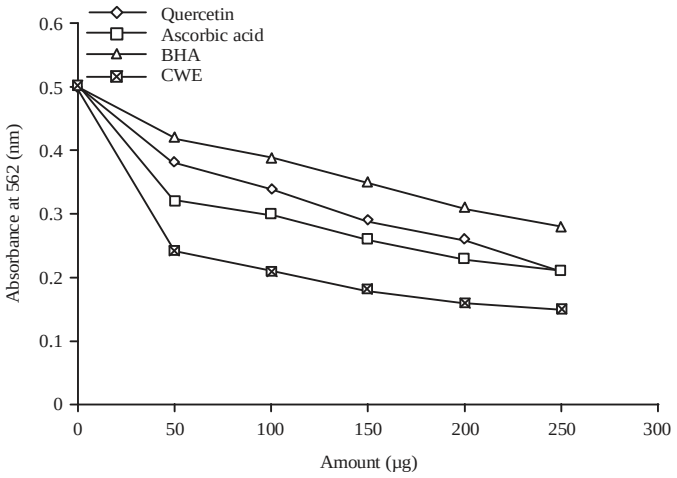

Fig. 5: Metal chelating effect of different amount of *Colubrina* water extract and standards on ferrous ions

activity which capture ferrous ion before ferrozine. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose-dependently (from 50-250 µg) (Fig. 5). The difference between CWE and the control was statistically significant ($p<0.01$). The percentages of metal chelating capacity of 250 µg concentration of CWE, ascorbic acid, BHA and quercetin were found as 84,40, 58 and 34%, respectively. The metal scavenging effect of CWE and standards decreased in the order of CWE>ascorbic acid>quercetine>BHA metal chelating capacity is important since it reduced theconcentration of the catalysing transition metal in lipid peroxidation (Duh *et al*., 1999). The data obtained from Fig. 5 revealed that CWE demonstrate a marked capacity for iron binding, revealing that their action as peroxidation protector may berelated to its iron binding capacity. Hydrogen peroxide scavenging activity of CWE may be endorsed to their phenolic content, which could bestow electrons to H_2O_2, thus counter acting it to water.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Arulmozhi *et al*., 2008). Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water (Nabavi *et al*., 2008; Ebrahimzadeh *et al*., 2009). The ability of CWE to scavenge H_2O_2 was determined according to the method of Ruch *et al*. (1989). The scavenging ability of CWE on H_2O_2 is shown in Fig. 6 and compared with BHA, BHT and ascorbic acid as standards. The CWE was capable of scavenging H_2O_2 in a dose-dependent. Two hundred and fifty micrograms of CWE exhibited 20% scavenging activity on H_2O_2. On the other hand, at the same concentration; BHA, BHT and ascorbic acid showed 32, 80 and 51% activity, respectively. These results indicated thatCWE posses potent H_2O_2 scavenging activity but had lower than the BHA, BHT and ascorbic acid. However, statistically significant correlation between those valuesand control ($p<0.01$) was observed. The H_2O_2 scavenging effect ofsame dose (250 µg) of CWE and standards decreased in the order of BHT>ascorbic acid>BHA>CWE.

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities (Nabavi *et al*., 2009). The 25.3 µg gallic acid equivalent of phenols was detected in 1 mg of CWE.

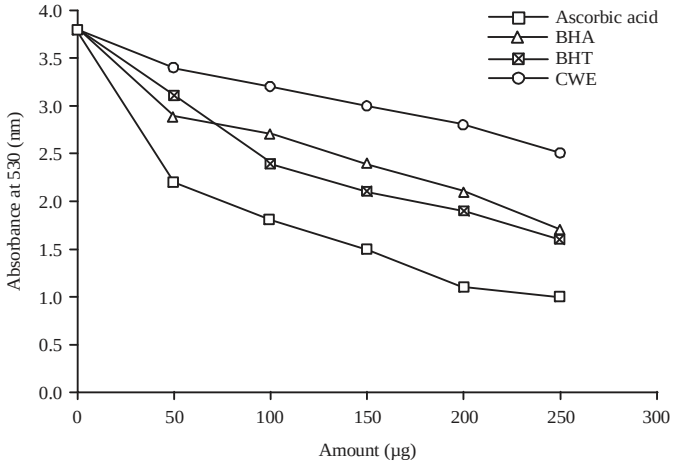

Fig. 6: Hydrogen peroxide scavenging activity of different amount of CWE, BHA, BHT, quercetin and ascorbic acid

Table 1: Composition of essential oil in *C. asiatica* leaves

Peak	Rt	Compound name	Percentage
1	13.4	Dodecamethylcyclohexasiloxane	19.0
2	20.1	Tetradecamethyl-cycloheptasiloxane	14.3
3	16.2	α-cubebene	14.0
4	2.5	2,4-dimethylhexane	13.0
5	5.3	6,6-methylenebicyclo[3.1.1]heptane	12.0
6	21.9	Cadina-1(10),4-diene	6.2
7	27.1	Hexadecamethyl-cyclooctasiloxane	4.9
8	6.6	Octamethylcyclo-tetrasiloxane	4.1
9	18.1	Isocaryophillene	3.2
10	6.9	Dehydro-N-[4,5-methylenedioxy-2- nitrobenzylidene]-tyramine	2.3

The phenolic compounds may contribute directly to theantioxidative action (Duh *et al*., 1999). The interest on these compounds is related with their antioxidant activity and promotion of health benefits (Ryan *et al*., 2002).

Essential oil composition and antibacterial efficacy: In the present study, the components present in the essential oil are identified using NIST library. The composition of essential oils showed dodecamethylcyclohexasiloxane the highest (18%) and decamethylcyclopentasiloxane showed the lowest percentage (1.6%) (Table 1). These compounds were reported to be in many personal care products such as toiletries. Volatile compounds is essential to determine the predominant components and their composition in order to investigate their bioactivity including antioxidant and antibacterial activities. A number of reports have shown that plant volatile compounds exhibited potent antioxidant and antibacterial activities (Choi and Hwang, 2005). Cubebene was reported to show potent antibacterial properties (Prabuseenivasan *et al*., 2006) and antioxidant properties (Ruberto and Baratta, 2000). Essential oils produced by plants have been traditionally used for respiratory tract infections and are used nowadays as ethical medicines for colds (Federspil *et al*., 1997). In this study, three different microbial and one yeast species were used to screen the possible antimicrobial activity of *Colubrina* Essential Oil (CEO). In the present study, 4 strains of bacterial strains representing gram negative and gram positive were used to screen the possible antimicrobial activity of CEO. Water extract of *Colubrina* exhibited antimicrobial activity against all tested microorganisms. Amongst these *Staphylococcus aureus*

Table 2: Antimicrobial activities of CEO (250 µg per disc) and standard antimicrobial agents

Tested microorganisms	Diameter of zone of CWE (mm)	Antimicrobial agents (mm)	
		Netilmicin (25 µg/disc)	Amoxicillin-clavulanic acid (15 µg/disc)
Escherichia coli	8	18	21
Pseudomonas aeruginosa	-	-	7
Staphylococcus aureus	9	17	13
Streptococcus pneumoniae	10	14	21

CEO: *Colubrina* essential oil, ND: Not detected activity at this amount of CWE or standards

gram positive bacteria responsible for food poisoning. Interestingly CEO showed antibacterial activity against this bacterium. Table 2 showed appreciable inhibitory activity of CEO. Escherichia coli, belonging to the normal ora of humans, is a gram negative bacterium. Amoxicillin-clavulanic acid (15 µg per disc) and netilmicin (25 µg per disc) were used as positive controls. Gram positive bacteria are known to be more susceptible to essential oils than gram negative bacteria (Smith-Palmer *et al*., 1998). Gram positive bacteria are more sensitive to plant oils and extracts than gram negative bacteria (Karaman *et al*., 2003).

CONCLUSION

Present findings clearly indicate that CWE has a powerful antioxidant activity against various oxidative systems *in vitro*; moreover, CWE can be further used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The various antioxidant mechanisms of CWE may be accredited to strong hydrogen donating ability, a metal chelating ability and their effectiveness as scavengers of hydrogen peroxide, superoxide and free radicals. The essential oil of *Colubrina* possessed noticeable antimicrobial activity against gram positive and negative bacteria when compared with standard and strong antimicrobial compounds such as amoxicillin clavulanic acid and netilmicin. We believe that the present investigation together with previous studies provide support to the antibacterial properties of *Colubrina* essential oil as well as potent natural antioxidant source. It can be used as antioxidant and antibacterial supplement in the developing countries towards the development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent and natural antioxidant compounds with health benefits in topical or oral applications.

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(III)

LYCOPODIELLA CERNUA (L.) PIC. SERM.: AN EXTENDED DISTRIBUTIONAL RECORD FOR NORTHERN WESTERN GHATS OF MAHARASHTRA

The genus *Lycopodiella* is commonly known as "Staghorn clubmoss". Generally it is distributed in moist and temperate tropics of the world. In India except some dry regions of Deccan and Indo- Gangetic plane more or lesley found in all states. It is commonest in South Indian moist forests and Himalayan foot hills region, North-East India also common. There is no authentic documentation was found on the occurrence of *Lycopodiella cernua* (L.) Pic. Serm in the state Maharashtra, Hence it is an extended distribution for Northern Western Ghats of Maharashtra, India.

Uses: It is cultivated as an ornamental plant. In Malaysia, decoction of the plant is used as a lotion in beri-beri and also for coughs and uneasiness in the chests (Manickam and Irudayaraj, 1992).

Lycopodiella cernua (L.) Pic. Serm., Webbia, 23 :166 (1968); Ollgaard, Opera Botanica, 92:172 (1987).

Lycopodium cernuum L., Sp. Pl. 2 : 1103 (1753). *Lycopodium capillacium* Willd., Sp. Pl. 5 : 31 (1880). *Palhinhaea cernua* (L.) Franco Et Vasc. In Vasc. Et Franco, Bot. Soc. Broter. Ser. 241 : 25 (1967): 213 (1982).

Terrestrial with long creeping or looping main stems (stolons) up to 1.8 (–5 in other areas) m long, bearing erect or less often procumbent stems at intervals 0.35–1(–2) m long. *Roots* short, up to 10 mm long. Stem erect much branched, densely leafy. *Leaves* are subulate, 1.5–5 mm long, 0.2–0.3(–1) mm wide, spreading, curved forwards. Strobili solitary at ends of leafy branchlets, 4–15 mm long, 2mm wide, sessile. *Sporophylls* isomorphic, pale yellowish, broadly ovate-acuminate, 1.8 mm long, 1.2 mm wide with lacerate-ciliate margins.

Distribution: Northeast India, Uttarakhand, Maharashtra. Africa, South America, Germany.

Current locality: N 15 52‘ 17. 29“ E 073 46‘ 56.06“. 108 m from Mean sea level along the sides of rail line near Sawantwadi railway station, the specimen was collected by authors during September, 2016 and deposited at Department of Botany, Shivaji University, Kolhapur.

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PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF THE LEAF EXTRACTS OF *CHROMOLAENAODORATA* L.

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Abstract : The present study was aimed to carry out the detailed phytochemical analysis of the leaves of *Chromolaenaodorata*. Qualitative phytochemical screening of the aqueous extract of fresh and leaf litter leachate extracts of the leaves revealed the presence of many components such as alkaloids, carbohydrates, flavanoids, reducing sugars. GC-MS analysis was also carried out to detect the phyto constituents present in both extract of *Chromolaenaodorata*.

Keywords: Chromoleanaodorata, Phytochemical screening, GC-MS. Leaf extract

INTRODUCTION:

Chromolaenaodorata (Linn) R.M. King & H. Robinson (Asteraceae) (syn. Eupatorium odoratum L. or Osmiaodorata L.) known as Siam weed is a fast-growing perennial, diffuse and scrambling shrub native to Central and Southern America then introduced into the tropical regions of Asia, Africa and the Pacific where it is an invasive weed. Plant grows to 3 to 7 m in height when growing in the open, and it goes by many common names including devil weed, French weed, communist weed, hagonoy, co hoy etc. (Ngozi et al., 2009; Chandrasekaran and Swamy, 2010; Vaisakh and Pandey, 2011; Kouamé et al., 2013; Otarigho and Morenikeji, 2013). This invasive weed is introduced to many places, either intentionally as an ornamental plant or accidentally, it is now regarded as one of the most harmful weeds present on earth due to its highly invasive and allelopathic nature (Vaisakh and Pandey, 2011; Otarigho and Morenikeji, 2013). This weed suppress crops and other plants in its surroundings by competing for nutrients and water, over-shading and allelopathy (Wilson, 2011). The younger leaves of *C. odorata* are toxic due to high levels of nitrate (Orapa et al., 2002).

In a preliminary survey, it was marked that a vast area of land, especially in the roadside of coastal area of Sawantwadi, Maharashtra are infested with the weed. The literature on the effect of Siam weed or its extracts on the growth of other coastal plants is quite scanty. Keeping in mind the above background, the present study was undertaken with the following objective of identification of allelo chemicals through GC-MS analysis of both fresh leaf extract and leaf litter leachate extract of *Chromolaenaodorata* L.

MATERIALS AND METHODS :

Preparation of extract

The fresh leaves of Insect-free, disease-free plants of *C. odorata* and Fallen matured senescent leaves were collected from the old plants growing were collected from the coastal area from Sawantwadi, Maharashtra, where it was growing abundantly. They were washed thoroughly with distilled water and air-dried at room temperature for 96 h. Both, fresh as well as fallen matured leaves chopped into 1-cm long pieces, and were grated with mechanical grater. The ground plant was soaked in 1 L of water for 24 hr. The extracts were then filtered with muslin cloth followed by Whatman filter paper No. 1.

Phytochemical screening

The phyto-components of the fresh leaf extract and leaf litter leachate extracts of the leaves of *C. odorata*were qualitatively analyzed in detail as per the standard methods (Kokate, 2000, Harbone, 1999 and Tiwari et al., 2011).

GC-MS analysis for phytochemical compounds

Samples were analysed with a Hewlett–Packard (HP) 6890 gas chromatograph fitted with a Gerstel MPS2 auto sampler and coupled to a HP 5973 N mass spectrometer. The carrier gas was helium (BOC gases, Ultra High Purity), flow rate 1.2 ml min^{-1}. The oven temperature was started at 50°C, held at this temperature for 1min, then increased to 220°C at 10° min^{-1} and held at this temperature for 10 min. The injector was held at 200°C and the transfer line at 250°C. For quantification of the compounds, mass spectra were recorded in the Selective Ion Monitoring (SIM) mode using NIST library.

RESULTS AND DISCUSSION :

Qualitative phytochemical analysis of the fresh leaf extract and leaf litter leachate extracts of *C. odoara.* The results of qualitative phytochemical analysis of both extracts of *C. odorata* are given in Table 1. Results indicate the presence of many phyto-components in both the extracts.

Tab.1.Results of Qualitative Phytochemical Screening of aqueous extract of fresh and leaf litter leachate extracts of the leaves of *C. odorata* L.

Sr. No.	Constituent	Test	Results	
			FLE	LLLE
1	Alkaloids	Mayer's reagent	+	+
		Wagner's reagent test	+	+
2	Carbohydrates	Molish's test	+	+
3	Flavanoids	Alkaline reagent test	+	+
4	Tannins and Phenolic compounds	Ferric chloride test	+	+
		Lead acetate test	+	+
		Dilute iodine solution test	+	+
5	Reducing sugars	Fehling's test	+	+
		Benedicts test	-	+

(FLE: Fresh Leaf ExtractLLLE: leaf litter leachate extract +: Present -: Absent)

GC-MS analysis

Gas Chromatography and Mass spectral analysis of bioactive volatile compounds from leaf litter leachate and leaf extract of ***Chromolenaodorata*** is shown in Table 2. while the mass fragments are presented in Fig.1 and 2.It is evident from the table and figure that both extract are rich in bioactive volatile compounds. Among these volatile compounds some represents class of monoterpens, alkenes and alcohols. The major essential oil found in both the extracts (leaf litter lechate and leaf extract) are α-pinene, β-pinene, 1,8-cineole, camphene, cymene and linalool, and the fatty acids are hexanoic acid , lauric acid, decanoic acid and octanoic acid.

A more divers compounds are monoterpens than the other volatile terpenoid compounds. The biological activities of compounds are mainly depending upon the synergistic or additive effects of different concentrations. According to Vokou*et al.,* 2003, Volatiles from aromatic plants can cause a number of positive or negative eggects. The biogenic and/or anthropogenic sources are the main source for the emission of All volatile organic compounds from plants (Desai, 2011). Ciganek*et al.,* 2007 stated that many plants emit substantial amounts of phytogenic volatile organic compounds (PVOCs), such alkanes, alkenes, alcohols, aldehydes, ethers, esters and carboxylic acids.

Table: 2. Compounds detected in GC-MS/MS analysis in the leaf litter leachate and leaf extract of *Chromolenaodorata*.

Sr. No.	Mol. Wt.	Name of Compound	*LLLE* % Area of Peak	*LE* % Area of Peak
1	256	n-Hexadecanoic acid	27.25	18.53
2	390	1,2-benzenedicarboxylic acid	7.27	24.14
3	154	2-Decenal	4.16	5.08
4	112	2-Heptenal	3.42	6.56
5	338	Tetracosane	2.43	8.25
6	282	2-nonadecanone	1.70	9.34
7	152	2,4-decadienal	0.96	2.63
8	240	Cyclopentadecanone	16.14	
9	268	Octadecanal	8.84	2.1
10	102	2-Hexanol	3.94	
11	618	Tetratetracontane	3.29	2.8
12	228	Tetradecanoic acid	2.78	
13	254	Heptadecane	2.31	
14	296	Heneicosane	2.27	
15	492	Pentatriacontane	1.95	3.6
16	268	Oxirane	1.70	
17	249	9,12-Octadecadienoic acid		46.12
18	182	1-tridecene		11.76
19	282	Oleic acid	8.7	10.37
20	324	4,8,12,16-tetramethylheptadecan-4-olide		8.91
21	126	2-ocrenal	1.3	8.38
22	158	Dec-2-en-1-ol		7.45
23	168	2-undecenal		6.20
24	144	Octanoic acid	3.4	4.57
25	142	Nonanal	2.7	4.18
26	256	Eicosanoic acid		4.12
27	100	Hexanal		3.29
28	270	Hexadecanoic acid	3.4	2.48
29	210	1-pentadecene		0.96
30	252	1-Octadecene		0.77
31		α – pinene	2.8	2.3
32		Camphene	3.2	2.8
33		1, 8-Cineole	4.3	3.7
34		Cymene	2.9	
35		Linalool	1.8	1.6

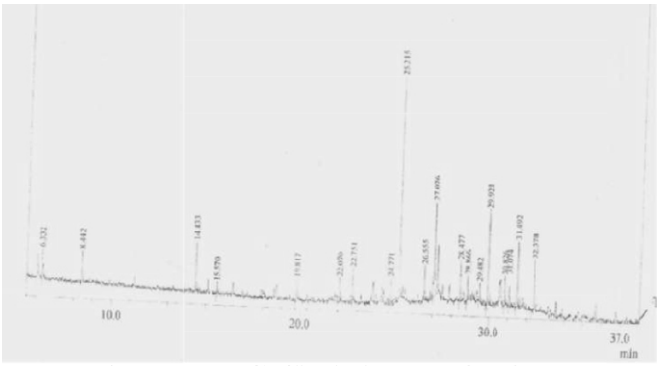

Fig. 1. GC Spectra of leaf litter leachate extract of *C. odorata*

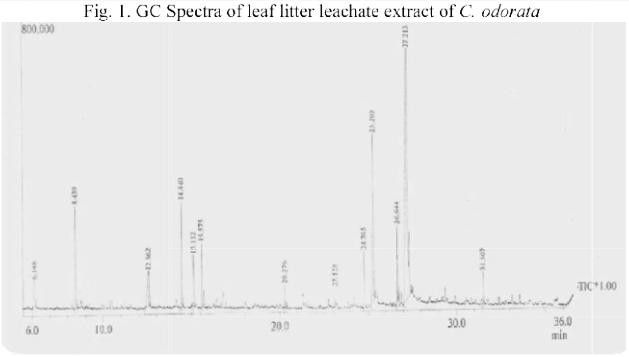

Fig.2. GC Spectra of leaf extract of *C. odorata*

Defense, communication or protection against adverse environmental conditions may be the reasons behind the emissions of volatile compounds (Niinemets*et al.,* 2004). Besides that, plant foliage is a natural passive sampling medium for collection of atmospheric dry or wet deposition of pollutants (Desai, 2011). There is a little information about toxicity of VOCs emitted in agriculture. Therefore, chemical identification and quantification is the necessary step in risk assessment of these compounds. (Ciganek*et al.,* 2007)

A complex blend of volatiles are released from plants in response to mechanical damages such includes injury caused by herbivore or microbial pathogens that give signal for herbivores and their natural enemies (Choudhary*et al.,* 2008). Several biochemical pathways are the origin of volatiles and the most commonly released volatiles include C6 volatiles (lipoxygenase/hydroperoxidelyase-dependent pathways), indole and MeSA (the shikimic acid/tryptophane pathway), cyclic and acyclic terpenoids (isoprenoid pathway), and oximes and nitriles (derived from amino acids) (Dicke, 1999). According to Choudhary and co-workers(2008), the release of terpenoid and C6 volatiles is strongly influenced by the emission of linolenic and linoleic acids from the membrane. These C18 fatty acids provide substrates for the synthesis of jasmonic acid (JA), C6 green-leaf volatiles (GLVs) or insect-modified lipid elicitors for volatile production.

The identified compounds from leaf leachate extract and leaf extract were active phyto components with allelopathic potential. According to the different structures and properties of these compounds, allelochemicals can be classified into the following categories: (1) water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; (2) simple unsaturated lactones; (3) long-chain fatty acids and polyacetylenes; (4) quinines (benzoquinone, anthraquinone and complex quinines); (5) phenolics; (6)

cinnamic acid and its derivatives; (7) coumarins; (8) flavonoids; (9) tannins; (10) steroids and terpenoids (sesquiterpene lactones, diterpenes, and triterpenoids) (Li et al., 2010). Thousands of chemicals are produced by plants for their self defense against herbivores, pests and even from neighbouring plants and compounds are released by plants in surrounding environment through volatilization, leaching, exudation and decomposition of plant residues (John and Sarada, 2012). According to Singh et al., (2003) the most common allelochemicals include cinnamic and benzoic acids, flavonoids and terpenes. While according to Einhellig, (2002) these compounds are phytotoxic. According to Zeng and its workers (2001), In allelopathy, phenolic compounds includes simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins and perhaps few flavonoids. According to Li et al., (2010), Phenolic compounds are a class of the most important and common plant allelochemicals in the ecosystem. They are chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Phenolic compounds are used in several industrial processes to manufacture chemicals such as pesticides, explosives, drugs and dyes. They are also used in the bleaching process of paper manufacturing. Apart from these functions, phenolic compounds have substantial allelopathic applications in agriculture and forestry as herbicides, insecticides, and fungicides (Santana et al., 2009).

According to Li et al., (2009), the impact of phenolic allelochemicals on the respiration of plants has mainly been shown to involve weakened oxygen absorption capacity, while the impact on photosynthesis has mainly been to reduce the chlorophyll content and photosynthetic rate. In view of John and Sarada (2011), Phenolic allelochemicals inhibits the plant root elongation, cell division, change the cell ultra-structure and subsequently interfere with normal growth and development of whole plant. Some phenolics (*i.e*., ferulic acid and cinnamic acid) can inhibit protein synthesis (He and Lin, 2001). According to All phenolics could reduce integrity of DNA and RNA (Zeng et al., 2001).

The allelopathicactivity of several fatty acids have been observed by Xian *et al.* (2005). According to Mishra (2015) the myristic acid and palimitic acid inhibits the plant growth. Geethambigai and Prabhakaran (2014) studied the allelopathic potential of *Cyperus* and *Cynodon*on germination and growth of some rice cultivars and found that the weed *C. dactylon* contains beta-sitosterol, beta-carotene, vitamin C, palmitic acid and triterpenoids. The allelochemicals present in the weed extracts might be jointly synergistic to seeds germination and seedling growth of rice.

According to Singh et al., (2001), Litter and soil from *Populusdeltoides*fields were rich in phytotoxic phenolics, their amount was more in fields, where parent soil was retained compared to fields replaced with soil collected from an area devoid of *P. deltoides*and control fields. The seedling length and dry weight of *L. culinaris*and *T. aestivum*were significantly reduced in litter amended soil. These observed reductions were attributed to the allelopathic interference of trees on crops through the release of phytotoxic phenolics from leaves and litter, which are continuously added to the soil. Espinosa *et al.* (2008) investigated the allelopathic potential of soil from Eucalyptus species (*E. grandis, E. urophylla*and *E. grandis*x *urophylla*) plantations on the germination and early growth of 4 crops [maize (*Zea mays*), bean (*Phaseolus vulgaris*), watermelon (*Cucurbitapepo*) and squash (*Citrullus vulgaris*)].

Sashikumar et al., (2001), Investigated the allelopathic compounds in leachates of bark, fresh leaves and leaf litter of *Eucalyptus tereticornis, E. camaldulensis, E. polycarpa* and *E. microtheca* revealed the presence of coumaric, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids and catechol. Desai and Gaikwad (2015) reported phytotoxic nature of leaf litter of mangrove *Excoeacariaagallocha* on rice crops.

CONCLUSION :

The leaves of *C. odorata*contain many important phytochemical components such as alkaloids, carbohydrates, flavanoids…etc. The GC-MS analysis also showed the presence of many bioactive components. The phytotoxicity of *Chromolenaodorata* in nature might be due to the interactions of various groups of fatty acids and phenols Further studies are needed to detect the presence of bioactive compounds in the leaves of *C. odorata*using various other solvents such as methanol, petroleum ether.

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Screening of bioactive compounds of Sesbania grandiflora and Pistia stratiotes

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Screening of bioactive compounds of *Sesbania grandiflora* and *Pistia stratiotes*

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ABSTRACT

The Sawantwadi region is under Konkan platue which is part of Western Ghats of India and known for its biodiversity. To know plant defense mechanism here is an attempt have been made in the form of preliminary phytopharmacological survey of the *Sesbania grandiflora* and *Pistia stratiotes* plants. The qualitative analysis of aqueous extracts of leaf and flower have carried out with reference to saponins, phenols, tannins, phytosterols, triterpens, alkaloids terpenoids…etc.

Key Words: Secondary metabolites, Phytochemical, *Sesbania grandiflora* and *Pistia stratiotes*.

INTRODUCTION

In Maharashtra, Konkan region famous for its biodiversity. Plants are integral part of nature. Most of the diseases are crop ups at humid condition. Sawantwadi region is under Konkan platue where there is always high humidity favorable for pathogenic attack. Although this climatic peculiarity for pathogens, they are unsuccessful to florish on the plants of this region. It may be due to strong chemical weapons developed by plant themselves. Plants have an almost endless variety of metabolites which is very useful to human beings (Suresh et al., 2011). Therefore to check this in present work an attempt have been carried out for retesting preliminary phytopharmacological survey of the *Sesbania grandiflora* and *Pistia stratiotes* plants.

Sesbania grandiflora L. is plant from family fabaceae cultivated in all over India for its edible flowers. It has synonym *Agati grandiflora* and commonly known as Hummingbird Tree, Butterfly Tree. It is small ornamental tree with a straight trunk produces white flowers like little birds. Bark, leaves, gums, and flowers have medicinal potential. Dried bark powder is used in cosmetics. An aqueous extract of plant is said to be toxic to cockroaches. Plant may produce some chemical constituent responsible for it. *Pistia stratiotes* is another plant from family Araceae is anciently used in Ayurvedic medicine. It is found in ponds and streams almost throughout India oftenly known as water cabbage, water lettuce, Nile cabbage and Jalkumbhi.

MATERIAL METHODS

Preliminary photochemical testing for the presence of various compounds by standard methods like Steroids, Benedict's test for reducing sugar, Hagers test , Mayer's test, Wagner's test and Dragendroff's test for Alkaloid, Tannins, Saponins, Terpenoids by Salkowski test (Robert et al.,2011; Parekh and Chanda, 2008; Gibbs, 1974; Wagner et al.,1996 and Treare and Evans,1985 respectively) and compounds like Phenols, Flavonoids, Quinons, Glycosides and Triterpenes compounds by Khandelwal (2002) were conducted. Molisch's test, Benedict's test and Fehling's test for carbohydrates.

Phytochemical screening:

Phytochemical examinations of *Sesbania* and *Pistia* were carried out for aqueous as per the standard methods.

Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Wagner's Test:

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's Test:

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test:

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test:

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test:

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of saponins

Froth Test:

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of phytosterols

Libermann Burchard's test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Triterpenes

Salkowski's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of phenols

Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of flavonoids

Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test:

To 1ml of the plant extract in a test tube was added 1ml of 5% lead acetate and the mixture was allowed to stand on the bench. The formation of precipitates in any of the samples showed that the extract contained flavonoids.

Test for glycosides

A quantity (20 ml of 50% H2SO4) was added to 2 ml of the concentrated leaf extracts in a test tube. The mixture was heated in a water bath for 15 min. A quantity (10 ml) of Fehling's solution was then added and the mixture was boiled. Development of a brick-red precipitate indicated the presence of glycosides in the extracts.

Test for steroids

Here, 5 drops of concentrated H2SO4 were added to 1 ml of the leaf extract. Development of red colouration was indicative of a positive reaction.

Test for Tannins

About 0.5 g each portion was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Test for Starch

The iodine solution was added to aqueous extract blue colouration indicate presence of starch.

Test for terpenoids (Salkowski test)

The extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for oxalic acid

Extracts was treated with aqueous CaCl2 solution appearance of precipitate indicates presence of oxalic acid.

Test For Quinone

Extracts was treated with concentrated HCl appearance of green colouration indicates presence of Quinone

Test for Triterpens

To 0.5 g each of the extract was added 2ml of chloroform concentrated H2SO4 [3ml] was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

RESULTS AND DISCUSSION

In recent years, secondary plant metabolites extensively investigated as a source of medicinal agents. It has been accepted that natural compounds play an important role in health care. Plants-derived substance have recently become of great interest owing to their versatile application. Plants are the richest bio-resource of traditional system of medicine, food supplements, folk medicine, and pharmaceutical intermediates. It is a well documented that the presence of these chemicals is responsible for various medicinal properties and reported time to time by various researchers.

It is evidence from result that *Sesbania grandiflora* and *Pistia stratiotes* both plant species shows positive reaction for secondary metabolites like alkaloids, starch, phytosterol, phenol, flavonoid, Steroids, Tannins, Oxalic acid, Triterpens and glycosides. Floral part of *Sesbania grandiflora* gives negative response to starch, phytosterol, terpenoids and glycosides. Vegetive part like leaves of *Sesbania grandiflora* shows abundance of all test of phytochemicals except carbohydrate for Molisch's Test, Benedict's Test, Fehling's Test but floral part shows satisfactory response to Benedict's Test and Fehling's Test. Pistia leaves shows presence of Alkaloids, Starch , Phytosterols, Phenols, Flavonoids ,Glycosides, Steroids, Tannins and Oxalic acid. While compounds like Carbohydrates, Saponins, Triterpenes, Terpenoids and Triterpens shows negative response as shown in table 1.

Tannins are secondary metabolites responsible for antimicrobial, astringency properties i.e. faster healing of wounds and inflamed mucous membrane in various plants (Chung, 1998 and Okwu, and Josiah, 2006). According to Singh et al. (2007) phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, as well as inhibition of angiogenesis and cell proliferation activities. Saponins have been extensively used as detergents, pesticides and molluscicides & also have beneficial health effects. This also shows property like precipitating and coagulating red blood cells (Shi et al., 2004). Phytosterol acts as growth hormones in plants. The plant has medicinal property due to presence of these phytochemicals.

CONCLUSION

From the above study, it is concluded that *Sesbania grandiflora* and *Pistia stratiotes* both containing some valuable secondary metabolites and it indicate their medicinal value due to the presence of phytoconstituents. These bioactive compounds make them useful for treating different ailment and have a potential of providing useful drugs of human use. This study supports usefulness of *Sesbania grandiflora* flower in food as vegetable. Thus the further work aiming towards tracing out of phytochemical present in it and pharmacological activities are in progress.

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Table No.1: Qualitative Phytochemical estimation of *Sesbania grandiflora* and *Pistia stratiotes*

Phytochemical Tests	***Sesbania grandiflora***		***Pistia stratiotes***
	Flower extract	Leaves extract	Leaves extract
Alkaloids			
Wagner's Test	+	+	+
Hager's Test	+	+	+
Carbohydrates			
Molisch's Test	-	-	-
Benedict's Test	+	-	-
Fehling's Test	+	-	-
Starch	-	+	+
Saponins			
Froth Test	+	+	-
Phytosterols			
Libermann Burchard's test	-	+	+
Triterpenes			
Salkowski's Test	+	+	-
Phenols			
Ferric Chloride Test	+	+	+
Flavonoids			
Alkaline Reagent Test	+	+	-
Lead acetate Test	+	+	+
Glycosides	-	+	+
Steroids	+	-	+
Tannins	+	+	+
Terpenoids			
Salkowski's test	-	-	-
Oxalic acid	+	+	+
Triterpens	+	+	-

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