II. REVIEW OF LITERATURE

The review of literature related to present investigation and other medicinal plants has been illustrated as follow.

2.1: Survey of medicinal plant diseases:

Sinha and Datta (2002) reviewed diseases of important medicinal plants with respect to etiology, disease symptoms, mode of infection, perpetuation and effective methods of management of different foliar diseases i.e., downy mildew, powdery mildew, leaf spots and blights. They also described the soil borne diseases like damping off, root rot and wilts.

Chavan and Korekar (2011) conducted a survey for medicinal plants fungal diseases in various Talukas of Osmanabad District, Maharashtra State, India during the years 2008 and 2009. In the present survey, they have collected commonly occurring economically important three medicinal plants viz. *Aloe vera* (L.) Burm. *Datura metel* L. and *Withania somnifera* (L.) They observed leaf spot, leaf blight and leaf rust diseases.

You (1994) studied the occurrence and control of sprout yellow and rot of ginger. He described the symptoms and control methods of sprout yellow rot of *Zingiber officinale*.

Kulkarni and Prashanthi (2002) reviewed several diseases of Holy basil (*Oscimum sanctum*), Mint (*Mentha spp.*) and piece (*Cephaelis spp.*). They described extensively symptomatology and management of diseases along with etiology and epidemiology.
Peikun, and Jiang (1994) collected different infected medicinal plants i.e, *Dioscorea opposite*, *Scrophularia ningpoensis*, *Rabdosia serra*, *Eupatorium fortune*, *Alisma platago-aquatica* and *Catharanthus roseus*. They reported the 7 new species and combination of *Cercospora* and allied genera of medicinal pants. They described new species as *C. cantonensis* on *Dioscorea opposite*, *C. scrophularicola* on *Scrophularia ningpoensis*, *C. rabdosiae* on *Rabdosia serra*, *C. eupatorii-fortuniei* on *Eupatorium fortune*, *C. alismatricoala* on *Alisma platago-aquatica*, *C. stahlianthi* on *Stahlanthus involucratus* and *Corynespora catharanthicola* on *Catharanthus roseus*.

2.2. Diseases:
The fungal diseases of different medicinal plants reported are as under.

Doshi and Sharma (2002) reviewed overall status of the diseases of some important medicinal plants such as Opium poppy, Withania, Rauwolfia and Aloe. They studied diseases of Withania, Rauwolfia and Aloe in relation to symptoms and management, as not much information is available for etiology, disease cycle and epidemiology.

Gupta, et. al. (2004) reported a new disease of Ashwagandha (*Withania somnifera*) as root rot and wilt caused by *Fusarium solani*. They observed the symptoms of root rot and wilt in the fields of Ashwagandha at Lucknow, India. Initial symptoms were withering and drooping of plants, while at later stages, plants showed sever wilting leading to death and decay of underground parts. They observed the pulpiness with brownish color in the root of infected plants. They found white cottony growth of the fungus at the basal part of infected plants near ground level. The Plants in nurseries also shows symptoms of yellowing, drooping and decay at seedling stage
leading to 30-50% mortality. They further investigated and identified of fungus as *Fusarium solani*. It is the first pathogen report of root rot and wilt of *Withania somnifera* caused by *Fusarium solani*.

Mehrotra and Thapar (1990) reported leaf spot and blight of *Rauwolfia serpentina* caused by *Rhizoctonia solani* (Anamorph of *Thanatephorus cucumeris*) for the first time in India, based on observations made in 1988 in an experimental plot at the New Forest, Dehra Dun, Uttar Pradesh. This disease was previously been reported on *Pinus kesiya* and several broad leaved species in Assam and Meghalaya. It caused premature defoliation in July and August when the rains were frequent and heavy. The fungus was isolated successfully and used for inoculation tests. This pathogen also attacked different weeds *Setaria glauca* (*S. pumila*), *Ageratum houstonianum* and *Bidens biternata* growing with *Rauwolfia serpentina* and weeding up to a distance of 45 cm effectively controlled it.

Sutare and Kareppa (2010) studied severity index of fungal diseases of *Adhathoda Zeylanica* Medic caused by *Alternaria alternata*, *Colletotricum capsici* and *Acidium adhatodae* in Parbhani and Nanded District. They found that disease severity is more in Parbhani as compared to Nanded district.

Joshi and Kareppa (2010) detected on the fungal diseases of *Chlorophytum borivilanum*. Baker (Safed musli). They collected the infected leaves from fields of Parbhani and Nanded districts of Marathwada region. They isolated, purified identified and performed the pathogen pathogenecity test. They identified the different fungal pathogens as *Alternaria alternat* causing leaf spot, *Colletotricum dematium* causing leaf spot and *Uromyces clingii* causing leaf rust.
Thyagarthi and Manchanahally (2013) studied the fungal foliar diseases of *Rauwolfia serpentina* in wild, its seasonal occurrence, seed transmission and disease management. They conducted survey of foliar diseases in *R. serpentina* in Bhadra, Wildlife Sanctuary during 2006–2009. They determined the foliar disease incidence and its distribution and disease severity in forest regions of the sanctuary. The seed-borne nature and transmission of the causal organism was also determined. They carried out the management of seed-borne inoculums by seed dressing with fungicides. They also determined the effect of foliage infection on secondary metabolite content. They found that *Cercospora rauwolfiae* is a major leaf spot disease causing pathogen. They also observed that the disease is homogeneously distributed through the study area. The foliar disease severity was high in Kagemanegiri forest during October–November. The minor leaf spot disease caused by *Alternaria alternata* occurred occasionally. *Alternaria alternata* is seed borne and seed transmitted and could be managed by seed treatment with Captra. They found that secondary metabolites like alkaloids and steroids decreased with increase in foliar infection by *C. rauwolfiae*, while phenol and flavonoid contents increased. This study suggested that wilted *R. serpentina* is affected by *C. rauwolfiae* and *A. alternata*. The latter pathogen is seed-borne and seed transmitted and can be controlled by seed treatment.

Varadarajan, (1964) observed the Anthracnose disease of *Rauwolfia serpentina benth*. They identified the causative agent as *Colletotrichum gloeosporioides* Penz. They found that this is a new host record of *C. gloeosporioides*. They observed that infected spots parts shows that enlarged to form larger circular patches, several of which coalesced, causing complete drying of the lamina and defoliation.

Datidar, Nigam and Kandalakar, (1993) observed selections for high dry root yield of Ashwagandha. They developed breeding material by crossing WS20 and WS22 with wild accessions i.e., evaluated in the F2 and F3 generations. Overall mean for dry root yield of 134 F3 lines was 20± 10.7 g at Mandsaur in 1989-90. They used the top 39 lines under a selection pressure of 29 %. They obtained dry root yields averaged 38.1 ± 6.6 g and 38.6 ± 11.5 gm in the selected lines from crosses involving WS20 and WS22 respectively.

Sadanandan, (1989) performed management of wilt affected black pepper (*Piper nigrum L.*) gardens in Kerala in India. The wilt of *Piper nigrum* is caused by *phytophthora palmivora*. They studied the agro-technology for the management of wilt affected by *Phytophthora palmivora* in gardens for 4 years. They found that management of the wilt affected pepper in gardens is economically feasible.

Plowman, Leuchthmann, et.al, (1990) observed the significance of the fungus *Balansa cyperi* infecting medicinal species of Cyperus belonging family Cyperaceae from Amazonia. They examined the herbarium specimen
collected throughout South America. They also collected living specimens in Ecuador and found that plants are infected by the systemic, ascomycetous fungus *Balansia cyperi*. They obtained pure cultures from 3 species i.e, *Cyperus articulates* and *Cyperus proixus* from Ecuador and *Cyperus virens* from southern USA, and grown in submerged liquid culture. They found several unidentified ergot alkaloids from the extract of liquid medium.

Purohit and Vyas, (2007) reported various diseases on *Rauwolfia serpentina* with their symptoms such as Anthracnose caused by *Colletotrichum gleosporioides*, Die-back caused by *Colletotrichum dematium*, leaf blight and bud rot caused by *Alternaria tenuis*, leaf spots caused by *Cercospora rauvolfae* and wilt caused by *Fusarium oxysporum f.sp.rauvolfii*. They also reported disease management of Rauwolfia diseases.

Capelli, Buonaurio and Polverar, (1991) studied common diseases of Saffron in Italy. They found damping off, basal stem rot, drooping and wilting of shoots in Saffron. They identified the causal organism as *P. corymbiferum*.

Pawar and Badar (2012) studied fungal diseases of some Ethnobotanical plants from Gautala Sanctuary Kannad,Maharashtra. They isolated fourteen fungal genera out of these eleven belongs to deuteromycetes, seven to ascomycetes and four to basidiomycetes.

2.3: Isolation:

Mukadam et. al., (2006) illustrated several pathogenic fungi. They isolated and studied the characters of *Macrophomina phaseolina*. 
2.4: Pathogenicity test

Ojha, et.al, (1988) observed the two new leaf spot diseases of Betel vine from India. They studied pathogenicity of isolated pathogen *Alternaria alternata* and *Curvularia lunata* from leaves of piper betel and confirmed their pathogenicity.

Ykema and Stutz, (1991) isolated *Fusarium equiseti*, *Fusarium oxysporum* and *Fusarium solani* from the necrotic root tissue of diseased guayule. They observed that *Fusarium solani* as the most prevalent among them. They proved the pathogenicity test and found that *Fusarium oxysporum* as the most virulent, causing 60-70% root rot symptoms of the inoculated roots.

Sidhu and Behl, (1991) studied the Blossom bight of *Farthenium argentatum* (guayule) in India. They found that blossom blight is caused by *Alternaria infectoria*. They isolated the fungus from blighted capitula and confirmed its pathogenicity. This is the first report of blossom blight of guayule caused by *Alternaria infectoria* and is also a first report of recovery from a dicotyledonous.

Taemitti and Matta, (1989) found the wilt of Holy basil in Liguria. The main symptoms of the disease are dwarfing, foliar wilting and xylem browning. They identified the causal agent as *Fusarium oxysporum* f. spp. *basiicum* and it has high optimum growth at temp. 30-32°C.

Ahmed, (1990) studied rusts and powdery mildews of some medicinal plants in Arunachal Pradesh, India. He reported first time *Uredo paederiae* causing rust of *Paederia foetida* in India. He also observed *Puccinia
oxalidis, Puccinia menthae and Coleosporium perillae causing rust of Oxalis corymbosa.

Housagodar and Balakrishnan, (1995) observed the diseases of Cinnamomum schaeffer (Lauraceae) in India. They prepared a descriptive key for the major diseases of cultivated and wild Cinnamomum spp., including 6 black mildews (Meliolaceae), 3 rusts (Uredinales), Exobasidium cinnamomi, die-back by Colletotrichum gloesporioides, Exosporium cinnamomi and Rosenschliella cinnamomi. They explained on the incidence, distribution and control of these diseases and their relative economic importance. They tabulated other minor diseases with their hosts and localities.

Cen Bingzhan and Deng Ruiliang, (1994) identified the pathogen causing Cinnamon dieback disease of Cinnamomum cassia (C. aromaticum) in China. They identified the pathogen as Botryodiplodia abromae. In single spore culture they observed, 204 out of 248 clones as grey and the remainder as black. They also studied the differences in pathogenicity, growth rate, reaction to boron, effects of light, pigment production and electrophoresis of protein and SOD and depending upon these studies they suggested that two distinct physiological races (A & B) are present.

Sholberg and Ginns (1996) first reported the powdery mildew on ginseng in North America. They observed powdery mildew on American ginseng (Panx quinquefolium) at Summerland, British Columbia, Canada in the summer of 1995. They noticed the diseases on a research plot where 3-year old plant was growing on raised beds under artificial shades. They found that upper surface of 5-10 leaflets of many plants were covered with
colonies of extensive, white, superficial mycelium. Leaves became yellow and fall prematurely. The fungus produced abundant cylindrical to oblong, arthroconidia of 40x14um size and arthroconidia borne singly on simple conidiophores, 60-110 um long and 8-8.5 um in diameter. They identified the fungus as an *Erysiphe* sp., because they observed 2 or more asci in each ascoarp and the appendages were hyphoid. This is the first report of powdery mildew on ginseng in North America. A specimen has been deposited in the National Mycological Herbarium, Canada.

Alam, Sattar, et. al., (1996) observed the damping off, a new disease of opium poppy. They found the causative organism as *Pythium dissotocum*. They described the incidence, symptoms and pathogenicity of damping off of *Papaver somniferum* caused by *Pythium dissotocum*, in U.P., India. They found that the disease is prevalent in Barabanki (40-60%) and at experimental fields at Lucknow (>60%). They observed that the affected seedlings turned yellow and the root and collar region decayed. They isolated the causal agent from diseased roots and identified as *Pythium dissotocum*.

Dorro, Sharma, et.al., (1994) studied the effect of organic amendments of soil on rhizome rot, nematodes and rhizosphere mycoflora of ginger (*Zingiber officinale Roscoae*). They reported that the incidence of rhizome rot of *Zingiber officinale* was minimum in soil i.e., treated by powder of Pinus needle and Neem cake. They found that melodies population was reduced to 74% and no Pratylenchus population was recorded in soil following any of the 4 treatments i.e., Neem cake powder, sawdust, Pinus needles or Quezcus leaves. They found that *Trichoderma* and
Gliocladium populations were maximum in Neem cake and Pinus needle treatments.

Rao, Sasikumar and George, (1995) detected the field reaction of ginger germplasm to Phyllostica zingiberi. They evaluated the ginger germplasm accessions (100) for their reaction and tolerance to Phyllostica zingiberi leaf spot under field conditions at the Research Farm., Pennuvannamuzhi, India. They developed a scale for severity of symptoms of infection in the infected plants. They also developed a disease index table for the 100 accessions and found that 11 accessions and tolerant and a further 42 were moderately tolerant.

Byadgi, (2002) presented an article which discussed about the diseases of medicinal plants like Anethum, Asparagus, Artemisia, Bergania, Boswellia, Calotropis, Crotolaria, Ginseng, Marjoram, Moringa, parsley, Phytolacca, Pyrethrum, Tephrosia, Terminalia and Valerian.

Paul and Singh, (2002) published a review on diseases of some important medicinal plants. In this article, they discussed information regarding diseases of plants like, Costus, Digitalis, Dioscorea, Seabuckthorn, Bunium, Saffron, Humurus, Saussurea and Fagopyrum.

Vijayan and Thomas, (2002) reviewed the management strategies of diseases of aromatic herbs. They confirmed experimentally the pathogenicity of the respective causal organism of Leaf blight of Horse radish (Armoracia rusticana) and rot of Marjoram (Marjorana hortensis) caused by Coletotrichum gloeosporioides, leaf spot of pepper mint (Mentha piperita) caused by Corunespora spp. and Fusarium spp. They also described various pathogenic diseases of herbal spices in India and abroad.
2.5: Phytocompound:

Dhavle, et. al., (2012) studied the effect of leaf extract of *Withania Somnifera* L. Dual on linear growth of *Colletotrichum capsici*. They found that as the concentration of *Withania somnifera* increases linear growth of *Colletotrichum capsici* decreases. They observed maximum inhibition as 8.00 at 2.5 % concentration.

Hina Ashraf and Arshad Javaid, (2007) evaluated antifungal activity of aqueous leaf extracts of *Azadirachta indica* A. Juss., *Melia azedarach* L. and *Toona ciliata* Roxb. (Meliaceae) against *Macrophomina phaseolina*. They isolated *Macrophomina phaseolina* from sunflower plants infected with charcoal rot disease and selected infected plant tissue showing charcoal rot characteristics and bearing fungal sclerotia. They cut tissue into 5 mm long and 2-3 mm thick pieces and then surface sterilized these pieces with 1% NaOCl solution for about 2 minutes i.e, followed by thorough washing with sterilized water. They transferred surface sterilized pieces to malt extract agar (MEA) medium in 90 mm diameter Petri plates and incubated in dark at 30±2 °C. They maintained the pure culture of *M. phaseolina* in refrigerator at 4°C. They selected fresh leaves of the three selected test tree species of Meliaceae i.e, collected from University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan during April 2008. They blended plant materials after thorough washing with sterilized water. They passed materials through a muslin cloth and then filtered by using Whatman No. 1 filter paper. They stored resultant 20% (w/w) stock solution at 4 °C in a refrigerator. They found that the Aqueous extracts of 5-20% concentrations of both *A. indica* and *M. azedaracta* significantly reduced biomass of *M. phaseolina* by 34 – 85% and 43 – 78%, respectively. On the contrary,
aqueous extracts of *T. ciliata* stimulated the growth of the fungus at all the tested concentrations.

Choudhari and kareppa, (2013) studied on the Identification of bioactive compounds of *Zingiber officinale roscoe* rhizomes through gas chromatography and mass spectrometry. They collected the fully mature *Zingiber officinale rhizomes* from Brahmpuri, Hingoli District, Maharashtra, India. The rhizomes were identified and authenticated at Department of Botany, Dnyanopasak College, Parbhani. They prepared the extracts of *Zingiber officinale rhizomes*. They removed the soil from the rhizomes, washed for several times with distilled water to remove the traces of impurities from the rhizomes. They dried the rhizomes dried at room temperature and coarsely powdered. They extracted the powder with 70% methanol for 48 hours using soxhlet apparatus. They stored the extract in refrigerator until used. They used extract containing both polar and non-polar bio component of the plant material. They identified 30 compounds of *Zingiber officinale Roscoe* rhizome from methanol extract and Zingiberene was found as major component.

Syeda Fakehha Naqvi, et. al., (2012) studied the antifungal potential of an allelopathic grass *Dicanthium annulatum* (Forssk.) Stapf. against *Macrophomina phaseolina* (Tassi) Goid. isolated from Cowpea (*Vigna unguiculata* (L.) Walp.) plants suffering from charcoal rot disease. They extracted different parts of this grass namely shoot, root and inflorescence in methanol. After evaporation of methanol they prepared different concentrations (0, 0.5, and 1.0… 3.0 g ml-1) of the extracts and studied their antifungal activity. In general, extracts of all the three parts exhibited
antifungal activity. However, they observed a marked variation in antifungal activity among the extracts of different parts of the test grass. They found that there was 7 to 51%, 29 to 71% and 33 to 81% reduction in fungal biomass due to different concentrations of shoot, root and inflorescence extracts of *D. annulatum*, respectively.

Kuberan, et. al., (2012) evaluated certain plant extract against *Glomerella cingulata* causing Brown Blight Disease of Tea. They tested aqueous crude extracts of 50 plants against *G. cingulata* *in vitro*. They isolated the *Glomerella cingulata* from infected leaves of tea plants by using potato dextrose agar (PDA) medium. They purified the fungus by single spore isolation technique and identified based on the morphological and cultural characterization. They maintained the culture on PDA. They collected total fifty plants and taken their various parts (Leaves, bark, buds, root, Rhizome and fruit) from Athikulam, Mugavoor and Valparai in Tamil Nadu. They washed these samples thoroughly with tap water and surface sterilized, later cut into small pieces. They prepared the Stock solution of all the plant species by soaking the crushed plant materials in sterilized water for 24h at room temperature (28-32°C) and filtered passing through triple layer of muslin cloth and finally through Whatman filter paper No.1. They prepared the concentrations of 10% W/V by adding appropriate quantity of sterile distilled water into stock solution. They mixed 5ml of extract with 20ml/plate sterile PDA medium for bioassay. The without plant extract medium was served as control. For each treatment they performed three replications. They inoculated these plates with 5mm disc of freshly grown pathogenic cultures of, *G. cingulata* and incubated at 28±1°C. They recorded the observations for antifungal activity 72 hours after incubation. They
calculated the fungal growth inhibition in diameter on an average in each treatment relative to its growth in control. They found that the extract of *Pongamia pinnata* provide 100% growth inhibition of *G. cingulata* at the concentration of 10% followed by *Syzigium aromaticum* (81.87%), *Acorous calamus* rhizome (80.62%), *Parthenium hysterophorus* (78.75%), *Citrus melon* (78.12%), *Ageratum conyzoides* (78.75%), *Allium sativum* (73.10%) and *Abutilon indicum* (71.15%). Whereas the extracts of *Piper nigrum*, *Cinnamomum xanthocarpum* and *Acorous calamus* leave at 10% were ineffective against the pathogen.

Fuhlbohm, et. al., (2012) isolated *Macrophomina phaseolina* from the roots of symptomless plants of 23 weed species found in Australian Mungbean fields. Eight of these species are new host records for the world, while 14 of the remaining 15 species are new reports in Australia. They found that Isolates of *Macrophomina phaseolina* from all weeds were pathogenic to Mungbean seedlings. They suggest that apparently healthy weeds infected by *Macrophomina phaseolina* may serve as alternative hosts of the pathogen in Australian grain production regions.

Dubey, et. al., (2009) studied combined effect of soil solarization and Neem products (leaf, bark and oil cake powders and Neem oil) amendments for the survival of *Macrophomina phaseolina* sclerotia in soil. They treated propagules of *Macrophomina phaseolina* with different Neem products gradually decreased with increase in duration of soil solarization as compared to control. They recorded minimum number of sclerotia/g dry soil in 1% Neem oil and 10% cake powder amended soil. They detected maximum viable sclerotia in unsolarized control soil followed by bark
powder amended soil. Soil solarization effectively caused a decline in propagules of *M. phaseolina* by 20% in comparison with unsolarized control soil after 30 days. However, the effectiveness of solarization got potentiated upon addition of different Neem products. Neem cake powder shows the most toxic effect in decreasing the survivability of test pathogen sclerotia. The bacterial population was higher in solarized soil as compared to unsolarized control soil. Moreover, the bacterial counts increased after addition of Neem cake powder in solarized soil. The fungal population was found to be almost equal in leaf and oil cake powders amended soils. Seedling emergence (%) of soybean in solarized and nonsolarized soils was similar but the total number of infected plants in solarized soil was lesser than unsolarized control. Combined effect of solarization and cake powder amendment minimized the number of infected plants by 60% and increased the seedling growth and biomass as compared to control.

Khajista, et. al., (2013) investigated the antifungal activity of *Calotropis procera* (aak) against the phytopathogenic fungus *Macrophomina phaseolina* which causes charcoal rot in various economically important crops. They applied different concentrations of leaf and stem methanol extracts viz. 1, 2, 3, 4 & 5% against *M. phaseolina in vitro*. They found Leaf extract more effective and showed significant antifungal activity as its 3% concentration 16.5% reduces the fungal growth. Methanolic extracts of *Calotropis procera* stem was promoting the growth of test fungus except 5% concentration. They observed that *Calotropis procera* leaf extract was effectively suppressing the growth of *Macrophomina phaseolina* in screening bioassays, so they subjected this for fractional guided bioassays. Methanolic extract of *Calotropis procera* leaves was partitioned with n-
hexane, chloroform, ethyl acetate and n- butanol. Minimum inhibitory concentration (MIC) of these fractions and commercial reference fungicide (72 %WP, Puslan) was evaluated against *Macrophomina phaseolina*. Different concentrations from (700 mg-1.36 mg mL-1) were used and data was recorded after 24 and 48 hrs. n- Hexane and synthetic fungicide were most effectively retard conidial germination with (1.36 mg mL-1) MIC. The other fractions were comparatively less antifungal.

Tandel, Sabalpara and Pandya, (2010) evaluated the phytoextracts of eleven plant species *in vitro* against *Macrophomina phaseolina* and they found that the extract of onion bulb produced maximum inhibition (98.14%) followed by extract of acacia, ginger, Neem, garlic and karanj. The sclerotal formation was also not taken place in all these phytoextracts. They tested the effect of plant extracts of various plant species *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *Macrophomina phaseolina*. They took healthy fresh leaves, bulbs and rhizomes, washed thoroughly with fresh water and finally rinsed with sterile distilled water. They cut fifty gram of either leaves or bulbs or rhizomes into small pieces and mixed in a grinder by adding 50 ml distilled sterile water. They filtered obtained extracts through double layered muslin cloth in 150 ml flasks and plugged. Then, the extracts were autoclaved at pressure 1.2 kg cm-2 for 20 minutes. Potato Dextrose Agar (PDA) medium was prepared and 100 ml was taken in 150 ml conical flasks, plugged and sterilized at 1.2 kg cm-2 for 20 minutes. They added autoclaved extracts individually in melted, cooled and sterilized PDA @ 10 percent v/v at the time of pouring in the petriplates containing phytoextracts and incubated at room temperature. A 5 mm disc of actively growing 5 days old pure culture of *Macrophomina phaseolina* was
inoculated. They made three repetitions for each treatment and medium without phytoextracts served as control. They recorded observations on colony diameter and statistically analyzed and percent growth inhibition was also worked out. They recorded sclerotial formation on the basis of scores.

2.6: Biological control:

Hesamedin Ramezani, (2008) evaluated the efficacy of four fungal bioagents viz., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma polysporum* and *Trichoderma viride* in vitro condition against the Egg plant root rot caused by *Macrophomina phaseolina* pathogen. He obtained pathogen and bioagents used in the present study from the Division of Mycology and Plant pathology, IARI, New Delhi, India. He used one week old culture of pathogen and bioagents maintained on PDA slants at 28±2°C for the present study. He determined the antagonistic activity of these bioagents by Dual Culture Technique and recorded percent growth of antagonists, pathogen and zone of inhibition as after 8 days of incubation. Among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 percent as compared to the minimum of 7.30 percent by *T. hamatum*. Soil application of talc-based formulation of *T. harzianum*, *T. polysporum* and *T. viride* effectively controlled the root-rot of Egg-plant under field condition.

Ushamalini, et. al., (1997) studied the effect of biocontrol agents and plant products on *Macrophomina phaseolina* causing charcoal rot of cowpea and other soil microorganisms. They found soil application of Neem cake @ 150 kg/ha and Farmyard manure reduced the charcoal rot incidence significantly and increased the yield. Both *Trichoderma viride* and *T.
*T. harzianum* recorded a root rot incidence of 17.0 and 17.6 per cent respectively as against 38.3 per cent in control.

Shamsur Ahman, et. al., (2002) coated Mungbean seeds with the conidial suspension of three *Trichoderma* spp. viz. *T. harzianum*, *T. hamatum* and *T. viride* in order to control seed-borne *Macrophomina phaseolina*. They evaluated the treated seed including control treatment in blotter and in pot with soil. All three species of *Trichodenna* spp. showed excellent control of seed borne *M. phaseolina*. Moreover, they increased germination significantly including production of robust seedlings. The seed coating with three *Trichoderma* spp. viz. *T. harzianum*, *T. hamatum* and *T. viride* proved as excellent control of seed-borne *M. phaseolina* in Mungbean. On the basis of this investigation these three *Trichoderma* species can be recommended for controlling seed-borne *Macrophomina phaseolina* in Mungbean.

Vinod Kumar, et.al., (2007) tested the effectiveness of plant growth promoting rhizobacteria especially *Pseudomonas fluorescens* isolates against charcoal rot of chickpea both in green house as well as in field conditions. Most of the isolates reduced charcoal rot disease and promoted plant growth in green house. They found marked increase in shoot and root length in *P. fluorescens* treated plants. Among all the *P. fluorescens* isolates they found PF4-99 most effective in the improvement of chickpea crop in green house as well as in field. PF4-99 effectively promoted plant growth and produced indole acetic acid in culture medium. This isolate also inhibited the mycelial growth of the *M. phaseolina* under *in vitro* conditions and reduced the disease severity. Potential isolate (PF4-99) also significantly
increased the biomass of the chickpea plants, shoot length, root length and protein content of the chickpea seeds. A part from these, they also found enhancement in the total number of seeds per plant and their weight. The colonization of PF4-99 reduced the incidence of seed mycoflora by which indirectly enhanced the seed germination and vigorous index of seedlings. These results revealed that isolate PF4-99 is quite effective to reduce the charcoal rot disease both in field and greenhouse and also increases seed yields significantly. Therefore, this isolate appears to be an efficient biocontrol agent against charcoal rot disease as well as yield increasing rhizobacteria.

Muhammad Anis, et. al., (2010) tested in vitro the effect of biological control agents by seed treatment. In the present study they evaluated, efficacy of microbial antagonists viz., Aspergillus flavus Link, Paecilomyces variotii Bainier, Trichoderma viride Pers., Rhizobium meliloti Dangeard and Bacillus subtilis Ferdinand Cohn for their effect on plant growth promotion and against Macrophomina phaseolina (Tassi) Goid., the cause of root rot of sunflower (Helianthus annus L.). They obtained biocontrol fungi and bacteria from Mycological Culture Collection of Department of Botany, University of Karachi. The biocontrol agents viz., Aspergillus flavus Link, Paecilomyces variotii Bainier and Trichoderma viride Pers., and test pathogen Macrophomina phaseolina (Tassi) Goid. were inoculated simultaneously side by side on single Petri plate containing PDA medium supplemented with penicillin and streptomycin @ 200mg /L. Three replications were maintained for each biocontrol agent treatment and were incubated for six days. The efficacy of Rhizobium meliloti Dangeard and Bacillus subtilis Ferdinand Cohn were tested by streaking the bacteria at one
side of the Petri plate opposite to the test pathogen. The growth of the fungus was inhibited when it grew towards the bacterial colony on PDA. The inhibition zone was measured from the edge of test fungal mycelium to the edge of the bacterial colony after 6 days of incubation and expressed as percent inhibition over control. The effect of antagonists (fungi and bacteria) was tested by blotter paper method, to assess their effect on germination and biomass of sunflower seeds. Seeds were first soaked in the suspension of *M. phaseolina* (hyphae and sclerotia) followed with suspension of the antagonists separately, rolled in moist blotter and incubated at room temperature (28 ± 5 C). The seeds soaked only in *M. phaseolina* suspension served as control. Five replications were maintained. After 10 days, the germination, radicle and plumule lengths were measured. The vigor index was calculated by multiplying germination percentage with the sum of radicle and plumule lengths.

The efficacy of biocontrol agents was also tested in test tubes to check their effect on germination and biomass of sunflower seeds. Seeds were first soaked in the suspension of *M. phaseolina* (hyphae and sclerotia) Viz. followed with suspension of the antagonists separately, planted in sterilized soil and incubated at room temperature. The seeds soaked only in *M. phaseolina* suspension served as control. Five replications were maintained. Ten days after the germination, shoot and root lengths and weight were measured. The vigor index was calculated by multiplying germination percentage with the sum of shoot and root lengths.

The effect of biocontrol agents was proved by pot experiment. Seeds of sunflower (*Helianthus annus* L.) were surface sterilized with 1% Ca (OCl)_2 for three minutes, rinsed thoroughly in sterilized distilled water and dried aseptically. The seeds were coated with microbial antagonists viz.,
Aspergillus flavus, Paecilomyces variotii, Trichoderma viride, Rhizobium meliloti and Bacillus subtilis separately by using 2% gum Arabic as sticker. Ten seeds after treatment with microbial antagonists were transferred in test tube containing 9 ml sterile distilled water. The test tubes were shaken and dilution series was made. Bacterial cells / seed or fungal conidia / seed were calculated by using the formula: No. of cells or conidia x dilution factor. Soil used for this experiment was obtained from Botany Dept. garden and passed through 2 mm sieve to discard particles. The soil used was sandy-loam (sand, silt, clay: 70, 19, 11%), pH range from 7.5-8.1 with 24% moisture holding capacity, total nitrogen 1.5%, total organic matter 24%. Soil had natural infestation of 1-3 sclerotia of M. phaseolina / g was found by wet sieving dilution technique. Seeds of sunflower were treated with 48 hrs old culture of R. meliloti and Bacillus subtilis and 7 days old culture of Aspergillus flavus, Paecilomyces variotii and Trichoderma viride by coating the seeds with 2% gum Arabic as a sticker. Five seeds were sown in pots containing 300 g soil. There were three replicates of each treatment and pots without antagonist and without seed coating material served as control. Pots were kept randomized in a green house and soil was kept @ 50% MHC. Germination was recorded after 10 days and plants were uprooted after 30 days. The observations such as root length shoot length and fresh weight of root and shoot and incidence of root infection caused by M. phaseolina were recorded. Data was analyzed and subjected to analysis of variance (ANOVA) following the procedure as given by Gomez and Gomez (1984).

They observed that in dual culture assays, all antagonists inhibited the growth of M. phaseolina. Rhizobium meliloti and Bacillus subtilis showed maximum inhibition in the growth of M. phaseolina. They also observed that seed treatments with tested antagonists in blotting paper, test tube and pot
experiments, did not show any detrimental effect on germination of sunflower seeds. On the other hand, in all the experiments seeds coating with antagonists proved effective in protecting sunflower seeds from root rot and significantly increased in root length and vigor index.

All the biocontrol agents tested were found to be effective in inhibiting the mycelial growth of *M. phaseolina* in dual culture technique *in vitro*. Among these the antagonists, *R. meliloti* was found significantly better in inhibiting the growth of *M. phaseolina* Viz. followed by *B. subtilis* and *Trichoderma viride*. However, after 10 days *T. viride* showed mycoparasitism and grown over Viz. the *M. phaseolina*. Seed treatment with antagonist did not have any adverse effect on germination of sunflower seeds by blotting paper method. It was found that a significant increase in radicle length of seeds coated with the antagonists compared to the seed coated with *M. phaseolina*.

The plumule lengths of the antagonist-coated seeds and control were at par with each other. Treatment of the antagonists showed a significantly higher vigour index than that of control. The radicle length and plumule length i.e. plant height was maximum in seed treated with *B. subtilis* followed by *Trichoderma viride* and maximum biomass was observed in seed treated with *R. meliloti*. In test tubes seed treatment with antagonist did not have any adverse effect on germination of sunflower seeds. There was a significant increase in shoot length of seeds coated with the antagonists compared to the seed coated with *M. phaseolina*. Maximum plant length was detected by treatment with *B. subtilis* Viz., followed by *R. meliloti*. Seeds treated with the antagonists showed a significantly higher vigour index than seed treated with *M. phaseolina*. Antagonist-coated seeds in pots containing naturally infested soil gave significant better germination percentage.
compared to the control. The shoot and root lengths of the seedlings from the antagonist-coated seeds were also significantly better than those of control. Plant length was observed maximum with _B. subtilis_ followed by _P. variotii_ and maximum plant biomass was observed with _B. subtilis_. Seed dressing with microbial antagonists efficiently controlled _M. phaseolina_ infection on sunflower.

El-Fiki, et. al., 2004 studied the effectiveness of most tested antagonistic fungi and bacteria particularly _Trichoderma harzianum, Chaetomium bostryoides_ and found significant reduction in disease incidence at seedling stage and maturity stage causing charcoal rot. They observed that _Trichoderma harzianum, Chaetomium bostryoides_ are effective in reducing disease incidence at seedling stage and maturity stage of charcoal rot. This treatment consequently increased healthy survived plants at the maturity stage. This effect might be attributed to the antagonistic action of the tested antagonistic microorganisms against _M. phaseolina_. The antagonistic microorganism(s) might suppress the activity of a plant pathogen through the enzymatic digestion of the pathogen cell walls and production of inhibitory volatile substances as found by Sankar and Sharma (2001).

Sankar and Sharma, (2001) stated that 2 out of 9 isolates of _T. viride_ evaluated in preliminary tests showed superior performance against _M. phaseolina_ i.e., the causal of charcoal rot in maize _in vitro_ as all the nine isolates produced inhibitory volatile substances.

**2.7: Phytochemical analysis:**
Dhruv et. al., (2004) estimated the Reserpine content from pharmaceutical products by spectrophotometric analysis. They recorded the absorbance at 510 nm. They found this method as simple, sensitive and economically viable.

Panwar and Guru,(2011) studied alkaloid profiling and estimation of Reserpine in *Rauwolfia serpentina* plant by TLC, HP-TLC and HPLC. They used different plant parts such as leaves, roots and callus. They found the highest reserpine content from the *in vitro* regenerated roots and least from the leaves.

Deshmukh et. al., (2012) carried out the phytochemical analysis of *Rauwolfia serpentina* and their different biological activities. They evaluated the presence and absence of indole alkaloids by TLC and HPLC methods. They used quantitative and qualitative determination of indole alkaloids. They reported the antiproliferative activity of *Rauwolfia serpentina* for the first time. They used the plant leaves and roots extract by using solvent like ethanol and obtained 11.27% quantity of crude extracts. They estimated the indole alkaloids by using the methods like TLC and HPLC that showed the presence of four different indole alkaloid derivatives like ajmalicine, ajmaline, yohimbine and reserpine in root extract of *Rauwolfia serpentina*. They further performed the quantitative determination of *Rauwolfia* alkaloids by spectrophotometric analysis and observed that Ajmalicine content is more in leaf extract as compared to reserpine; ajmaline and yohimbine are greater in root extract of plant. They performed antimicrobial activity with the help of well diffusion assay, MIC and MBC also they reported that root extract was good against the tested *S. typhii* and was
proved to be the better option for further drug development. The antiproliferative activity of ethanolic root and leaf extract of *R. serpentina* on cancerous HeLa cell line was tested and found that the leaf extract was more effective with the IC$_{50}$ value of 196μg/ml.

Srivastava, et. al., (2006) established a sensitive and reproducible reversed-phase high-performance liquid chromatography (HPLC) method by using photodiode array detection for the simultaneous quantitation of important root alkaloids of *Rauwolfia serpentina* i.e, reserpine, ajmaline, and ajmalicine. They used a Chromolith Performance RP-18e column (100 × 4.6-mm i. d.) and a binary gradient mobile phase composed of 0.01M (pH 3.5) phosphate buffer (NaH$_2$PO$_4$) containing 0.5% glacial acetic acid and acetonitrile. It was run at a flow rate of 1.0 mL/min with the detector operated at a wavelength of 254 nm. It was found that calibration curves linear over a concentration range of 1–20μg/ml ($r = 1.000$) for all the alkaloids. They also validated various other aspects of analysis (i.e., peak purity, similarity, recovery, and repeatability). They observed the recoveries for the three components as 98.27, 97.03, and 98.38, respectively. The detection limits were 6, 4, and 8μg/mL for ajmaline, ajmalicine, and reserpine, respectively, and the limits of quantitation are 19, 12, and 23μg/mL for ajmaline, ajmalicine, and reserpine, respectively. They found that this method is simple, reproducible, and easy to operate and most useful for the evaluation of *R. serpentina* alkaloids. They collected *Rauwolfia serpentina* roots from the experimental fields of the Central Institute of Medicinal and Aromatic Plants Lucknow, India and they deposited a voucher specimen of the plant material in the Gene Bank of this Institute. They used solvents of HPLC grade. The standards of ajmaline and
ajmalicine were purchased from M/S SRL Mumbai, India, reserpine through M/S Sigma (St. Louis, MO), Sodium dihydrogen ortho-phosphate from Glaxo (Mumbai, India), and HCl (35% GR) bought from Merck Mumbai, India. Double distilled water after filtering through a 0.45-μm filter was used. The instrument Shimadzu (Kyoto, Japan) LC-8A gradient HPLC was used in Viz. equipped with two LC-8A pumps and controlled by a CBM-10A interface module and 7725 I manual injector valve (Cotati, CA) in the HPLC analysis.

Arvind Kumar, et. al., (2011) studied the presence of various alkaloids in *Rauvolfia tetraphylla*. They used the methanol extract of leaves, stem and roots of *Rauvolfia tetraphylla* and yohimbine as standard. They carried out the compression by HPTLC. They performed the quantification and identification of yohimbine by WINCATS software with densitometric detection (Camag Scanner -3). This technique allowed different separation profiles that can be useful for phytochemical characterization of various parts of plants. They observed linear calibration ranges as 10-1000 g/ml for yohimbine and detected 6.11% Yohimbine only in the leaves. They validated the HPTLC methods successfully and applied to the quantization of yohimbine. They collected plant parts viz. root, stem, leaves and seed of *Rauvolfia tetraphylla* from Divya Nursery near Patanjali Yog Peeth, Haridwar in September 2008, authenticated and deposited the Voucher specimens in the herbarium of Divya Pharmacy. Each sample root, stem, leaves and seed of *Rauvolfia tetraphylla* were used for methanol extraction. 5 g of the root, stem, leaves and seeds were grinded to a homogeneous powder of 20 mesh sizes. They took these crushed materials in round bottom flask and pored 10 ml methanol to dissolve the each sample material and
Centrifuges each sample at 6000 RPM (Remi KKK-35579). The supernatant was collected and filtered from Whatman no. 4 filter paper for removal of organic solvent. The filtrate was ready for sampling to obtain the active phytochemical from the leaves of *Rauvolfia tetraphylla* by chromatography. The purified alkaloids obtained from *Rauwolfia tetraphylla* and *R. serpentina*, reserpine, perhaps, the one mostly used as tranquillizing agents. Yohimbine was the principal indole alkaloid derived from the bark of the yohimbe tree (*Pausinystalia yohimbe*). They obtained that a good amount 6.11% of yohimbine from the leaves of *Rauvolfia tetraphylla*. Yohimbine was absent in the stem, seeds and root of the plant *Rauvolfia tetraphylla*.

Shanbhag, et. al., (2011) developed a simple and accurate HPTLC method for the quantification of reserpine. They used HPTLC as a method of analysis to develop a standard procedure based on fingerprinting characteristics for the evaluation of homoeopathic formulations. Fingerprinting of the in-house mother tincture was considered as a standard with that of different marketed samples available from manufacturers of homoeopathic medicines in India. They used the HPTLC method quantitatively in terms of stability, repeatability, accuracy and calibration providing the utility in the analysis of the mother tincture. The authentic dried roots of *Rauwolfia serpentina* from Bafco, Noida were used to prepare the mother tincture. The Reserpine (C\textsubscript{33}H\textsubscript{40}N\textsubscript{2}O\textsubscript{9} m. p. 360°C, purity >97% w/w by TLC) was purchased from Natural Remedies, Bangalore. They used solvents absolute ethanol, HPLC water, toluene, ethyl acetate, diethyl amine, chloroform of analytical grade (MERCK Ltd.) The collected dried roots powder was prepared and 5 g of this powder was added the requisite amount of alcohol and water as specified in HPI and prepared
the standard mother tincture by the percolation method. The tincture was transferred to suitable glass container and stored for further study. They confirmed the decomposition of the analyte during application or development by two-dimensional chromatography so that the chromatogram did not show any extra fractions. They confirmed the repeatability of the method by scanning 15 tracks of 4 µl volume std. mother tincture. They observed the co-efficient of variation (CV) as 0.454 and calculated the percentage recovery of reserpine using the above method. They found the average recovery values as 96.6% to 104.37%, which confirms that the method is validated. The HPTLC Fingerprinting characteristics of *Rauwolfia serpentina* mother tinctures obtained from manufacturer (A1 to A6) and the in-house std. MQ (A) had been scanned at 225 nm wavelength.

Hareesh Kumar, et. al., (2010) studied important requirement for the evaluation of herbal drug including the estimation of active constituent by considering different factors like climate, altitude, rainfall and other conditions responsible for growth of plants may affect the content of active constituents. Collection of drug from different geographical sources can give useful conditions required for the production of maximum amount of secondary metabolites. The samples of *Rauwolfia* were collected from four different parts of southern India and developed the HPLC chromatogram for standard reserpine. They prepared methanol extracts of different samples. They subjected these extracts for HPLC analysis to find out the content of Reserpine for preliminary information about the conditions that may influence on production of active constituents. They observed the significant variation in the content of reserpine. They collected the plants of *Rauwolfia serpentina* L. from Trissur, (Kerala), Shimoga (Karnataka), Coimbatore
(Tamil Nadu), Tirupathi (Andhra Pradesh). They identified and authenticated *Rauwolfia serpentina* from Dr. Jawahar Raveendra, Botanist and Foundation for Revitalization of Local Health Tradition (FRLHT), Bangalore, Karnataka. They excised roots from the plants, washed with running tap water and dried in an oven at a temperature of not more than $60^\circ$C. They dried roots were coarsely powdered and subjected for extraction. They treated separately the 100 mg of the powder from each plant with ammonia solution and then extracted with methanol until the extracts were colorless. They combined the methanolic extracts of each plant separately and concentrated in a rotary evaporator, and dried in a vacuum oven.

Panda, et. al., (2012) stated that the position of *Rauwolfia serpentina* in industry is emerging. Reserpine is the first herbal constituent included in modern medicine system due to its high demand over the world market. This genuine plant is almost on the track of extinction and in future can be categorized as an endangered species. Therefore, the present study was attempted to search reserpine from other parts of *R. serpentina* and *R. tetraphylla* hence both the species can be explored for the isolation of bioactive reserpine and this commercial plant i.e., *R. Serpentina* can be minimized from over exploitations and extinction and also to establish the various Pharmacognostical parameters of Rauwolfia species from Estern Odisha for their correct identifications. They collected *R. serpentina linn.* and *R. tetraphylla* from the tribal belts of the Baipariguda forest of Koraput district in August and September, The plant was identified, confirmed and authenticated by the taxonomist Dr. N.K .Dhal, Institute of Minerals and Materials Technology Bhubaneswar, Orissa India ,Vide Voucher Specimen no. (V.N. no-16,507) was deposited. After authentification leaf, stem and
root were collected in bulk and washed under running tap water to remove adhering dirt and shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder. They used U.V spectrophotometer (Systronics 105) and Leica microscope (EZ-4D). Reserpine (standard) was procured from Sigma Aldrich, Bangalore and other chemicals used in this experiment (Methanol) were of analytical grade and obtained from Nice Chemical Ltd. Cochin, India.

The coarse powder was taken in Soxhlet apparatus and extracted successively with methanol for 72 hours. The mark of each extract was dried and used for extraction with successive solvent. The liquid extracts were concentrated separately under vacuum and resulting extracts were kept in desiccator until further use. They carried out chemical tests of all the Methanolic extracts for the qualitative determination of phytochemical constituents. They found that reserpine is present in leaf, stem and roots.

Madhusudanan, (2008) studied the applicability of a new mass spectrometric technique, DART (direct analysis in real time) for the analysis of the hairy root culture of Rauvolfia serpentina. They analyzed the intact hairy roots by holding them in the gap between the DART source and the mass spectrometer for measurements. They characterized the two nitrogen-containing compounds, vomilenine and reserpine, from the analysis of the hairy roots almost instantaneously. They confirmed structures of the identified compounds through their accurate molecular formula determinations. This is the first report of the application of DART technique for the characterization of compounds that are expressed in the hairy root cultures of Rauvolfia serpentina. Moreover, this also constitutes the first
report of expression of reserpine in the hairy root culture of *Rauvolfia serpentina*.

DART mass spectrometry was performed by using spectrometer of JMS-100 TLC atmospheric pressure ionization time-of-flight mass spectrometer (Jeol, Tokyo, Japan) fitted with a DART ion source. It was operated in positive-ion mode with a resolving power of 6000 (full-width at half-maximum). The orifice 1 potential was set to 28 V, resulting in minimal fragmentation. The ring lens and orifice 2 potentials were set to 13 and 5 V, respectively. Orifice 1 was set to a temperature of 100°C. The RF ion guide potential was 300 V. Data acquisition was from m/z 10 to 1050. The DART ion source was operated with helium gas flowing at approximately 4 L/min. The gas heater was set to 300°C. The potential on the discharge needle electrode of the DART source was set to 3000 V, electrode 1 to 100 V and the grid to 250 V. The sample as hairy root was positioned in the gap between the DART source and mass spectrometer for measurements.

Deshmukh, et. al., (2011) performed the simultaneous quantitative estimation of two biologically active compounds reserpine and arjunolic acid in Tensowert tablet by using high-performance thin-layer chromatography. They used the TLC aluminium plates precoated with silica gel 60F-254 of 0.2 mm thickness. The sample was dissolved in methanol and carried out the linear ascending development in twin trough glass chamber saturated with mobile phase Toluene: Ethyl-acetate: Diethylamine: Glacial acetic acid (6.5:5.0:1.5:0.5 v/v/v/v) and densitometry. They performed the determination of these compounds by TLC scanner (CAMAG) at 254 nm in reflectance/absorbance mode. They observed that the Rf value of reserpine and arjunolic acid as 0.42±0.03 and 0.14±0.02, respectively. They found
linearity in the concentration range of 200 ng to 1600 ng for both reserpine and arjunolic acid. The linear regression data for the calibration plots showed a good linear relationship with $r^2=0.998$ and 0.995 for reserpine and arjunolic acid, respectively. They validated the method according to the ICH guideline for accuracy, precision, recovery, robustness and ruggedness. They detected that the reserpine and arjunolic acid contents quantified from herbal formulation (Tensowert tablet) well within limits. Statistical analysis of the data showed that the method is reproducible and selective for the estimation of reserpine and arjunolic acid. The Reference standard of reserpine and arjunolic acid was obtained from Natural Remedies Pvt. Ltd. Bangalore and Spice Pvt. Ltd. Chennai, respectively. The Analytical grade of Toluene, Ethyl acetate, Diethyl amine, Glacial acetic acid and Methanol was purchased from Merck Chemical, Mumbai. They obtained the Stationary phase pre-coated silica gel aluminium plate 60F-254 from E. Merck (Germany). They procured aqueous extract of *R. serpentina* and *T. arjuna* from Konarck Herbal Ltd. Mumbai. They prepared the methanol extract sample of *R. serpentina* and *T. arjuna* extracts They accurately weighed 100 mg of *R. serpentina* and *T. arjuna* extracts dissolved in 8 ml methanol and refluxed for 30 min. They filtered the solution through Whatman filter paper no. 1 and diluted with methanol up to 10 ml i.e, 10 mg/ml. They also prepared the test solution of Tensowert tablet. They refluxed Tensowert tablets powder (1 g) with methanol and made up the volume to 10 ml (100 mg/ml). Pipette out 1 ml from the stock solution and diluted with methanol up to 5 ml (20 mg/ml).They prepared the standard stock solution by weighing 1mg of reserpine and arjunolic acid and dissolved in 2 ml methanol, sonicate and diluted with methanol up to 10 ml (100μg/ml).
Harisaranraj, Suresh and Saravanababu, (2009) analyzed Indian medicinal plants (*Rauwolfia serpentina* and *Ephedra vulgaris*) for their chemical composition, vitamins and minerals. They collected leaves for experiment from Kolli hills, Tamil Nadu, India. The plant materials (leaves) were identified and authenticated by Dr S. Saravana Babu, Botany Department, C.N. College, Erode, India. They air-dried leaves for 10 days and milled into powder with the aid of an electrical grinder and finally stored in airtight bottles before analysis. The major elements comprising calcium, phosphorus, sodium, potassium, magnesium and the trace elements of iron and zinc were determined. The alkaloid determination was carried out by weighing 5 g of the sample it was added into a 250 ml beaker and added 200 ml of 20% acetic acid in ethanol and covered to stand for 4 h. This was than they filtered and the extract was concentrated using a water bath to one-quarter of the original volume. They added the concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete. After that they allowed the whole solution to settle and they collected the precipitate by filtration and weighed. The saponin determination was carried out by using the ground samples. 20 g of each plant samples added in 200 ml of 20% ethanol and heated the suspension over a hot water bath for 4 h with continuous stirring at about 55°C. They filtered the mixture and the residue re-extracted with another 200 ml of 20% ethanol. They reduced the combined extracts to 40 ml over water bath at about 90°C. It was transferred to concentrate into a 250 ml separator funnel and added the 20 ml of diethyl ether and shaken vigorously. They recovered the aqueous layer, while the ether layer was discarded and 60 ml of n-butanol was added. They washed the combined n-butanol extracts twice with 10 ml of 5% aqueous sodium chloride. They heated the remaining solution in a water bath and
after evaporation; the samples were dried in the oven to obtain constant weight. They calculated the saponin content in percentage. The results revealed the presence of bioactive constituents comprising alkaloids (1.24 to 1.48 m g/100 g), saponins (1.46 to 1.72 m g/100 g), flavonoids (1.46 to 1.86 m g/100 g), phenols (0.06 m g/100 g) and tannins (0.04 to 0.5 m g/100 g). The medicinal plants contained ascorbic acid (26.42 to 44.03 m g/100 g), riboflavin (0.20 to 0.42 m g/100 g), thiamine (0.11 to 0.18 m g/100 g) and niacin (0.02 to 0.09 m g/100 g). These herbs are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn. The importance of these chemical constituents is discussed with respect to the role of these herbs in ethnomedicine in India.

Hema Lohani, et. al., (2011) studied High Performance Thin Layer Chromatography Viz. developed for quantification of Reserpine in *Rauwolfia* and its allied preparations. They found it as rapid and accurate method. The method proposed was highly precise, sensitive, specific and reproducible with an average recovery of 78%. The limit of quantification was observed to be 112 ng. They collected the Whole plant of *Rauwolfia serpentina* from Haldwani district Nainital (Uttrakhand), and species were authenticated by Botanical Survey of India northern circle Dehardun Uttarakhand (India). The voucher specimens have been kept in the Institute (Centre for Aromatic Plants, Selaqui, Dehradun, Uttarakhand, India). They used all the solvents of AR (Analytical Reagent) grade. They procured the reference standard of Reserpine from Sigma Aldrich USA. Chromatographic conditions consists of HPTLC system equipped with a sample applicator device Camag Linomat 5. Camag twin trough chamber, Camag TLC scanner and integration software (Wincats) HPTLC Plate: Silica gel GF254 (Merck) 20 X 10 cm Mobile Phase The plate was developed in Solvent system
Chloroform: Methanol: Ammonia in previously saturated twin through chamber.

They found that the roots of this species are mainly explored rather than other parts. The analytical HPTLC data revealed that Reserpine is present in leaf, stem and root of both the species. Hence other parts of both the species can be explored for the isolation of bioactive reserpine. Further the commercial plant *R. Serpentina* can be minimized from over exploitations and extinction.

Hareesh Kumar, et. al., (2010) stated that *Rauwolfia serpentina* is medicinally famous herb in Ayurvedic and Western system of medicine. Reserpine is the important constituent of Rauwolfia Viz. is an indole alkaloids reported to possess anti hypertensive and tranquilizing activity. They developed High Performance Thin Layer Chromatography for detection, monitoring and quantification of Reserpine in Rauwolfia and its preparations. They found it accurate and rapid. They studied percent recovery by adding the different known amounts of standard reserpine to the sample before sample preparation. The average recovery was found as 98.78%. The newly developed HPTLC method is quick and reliable for quantitative monitoring of reserpine in Rauwolfia species. This proposed method was precise, sensitive, specific and reproducible. They observed the limit of quantification to be 40ng, and C.V% <2.3%. They found the Rf value of Reserpine as 0.43 and calibration curves linear in the range of 500 to 900 ng.

Aqsa Aslam, et. al., (2010) reported the antifungal activity of plant diffusates from 5 indigenous medicinal plant species of Potohar region viz., *Adhatoda zeylanica, Azadirachta indica, Capparis decidua, Dodonaea*
viscosa and Salvadora oleoides. They tested the antifungal activity against 3 pathogens attacking commercial crops viz., Alternaria solani, Rhizoctonia solani and Macrophomina phaseolina. It was observed that all selected medicinal plants exhibited considerable distinction in radial mycelial growth of tested pathogens. In all Dodonaea viscosa appeared significantly the most effective and suppressed the radial mycelial growth of the Alternaria solani and Rhizoctonia solani, on the otherhand, Adhatoda zeylanica exhibited maximum inhibition (77.44%) against Macrophomina phaseolina but Salvadora oleoides exhibited minimum inhibition against all tested pathogens. They also found that radial mycelial growth of selected pathogens reduced at an increase of plant diffusates concentration. Of the five different concentrations of plant diffusates, the highest inhibition in radial mycelia growth of all 3 pathogens was observed at 100 and 200g/l respectively, as compared to control, while minimum inhibition was recorded at 10g/l in all plant diffusates. The present investigation suggested that Dodonaea viscosa can be utilized for the management of fungal diseases caused by Alternaria solani, Macrophomina phaseolina and Rhizoctonia solani.

Reeta Kumari, et. al., (2013) stated that Rauvolfia serpentina is an important medicinal plant in the pharmaceutical world due to the presence of its immense therapeutic properties. The plant is known for curing various disorders because of the presence of alkaloids, carbohydrates, flavonoids, glycosides, phlobatannins, phenols, resins, saponins sterols, tannins and terpenes. The plant parts, root and rhizome have been used since centuries in Ayurvedic medicines for curing a large number of diseases. The plant contains more than 50 different alkaloids which belong to the monoterpenoid indole alkaloid family. The major alkaloids are reserpine,
ajmaline, ajmalicine, ajmalimine, deserpidine, indobine, rescinnamine, rescinnamidine, serpentine, serpentinine and yohimbine.

The herbal medicine is still the basis of primary health care for 75–80% of the world population because of its cultural acceptability, better compatibility with the human body and lesser side effects. Therefore, there is a need for us to search alternative, naturally available remedies for curing millions of people worldwide.

Gawade and Fegade, (2012) reviewed the reserpine as a potential antihypertensive agent. They stated that the root of *Rauwolfia serpentina* Benth has been used in India from century. The genus name was selected in honor of Dr. Leonhard Rauwolf, a 16th century German botanist, Physician & explorer. *Rauwolfia serpentina* is a large climbing herb or shrub, belonging to family Apocynaceae and found in the Assam, Pegu, Himalayas, Java, Tennasserim, Deccan, Peninsula, Bihar and the Malay Peninsula. Reserpine is the principle alkaloid of *Rauwolfia serpentina* and has its clinical application with success to the treatment of high blood pressure. Much smaller dose of Reserpine is required to obtain the antihypertensive action.

Atsu Koh Itoh et al., (2005) isolated five new alkaloids from dried roots of *Rauwolfia serpentina* Viz. methylajmaline, methylisoajmaline, hydroxysarpagine, yohimbinic acid and isorauhimbinic acid.

Paul, et. al., (2006) studied the influence of soil moisture on physiological characters and root alkaloid yield of *Rauwolfia serpentina*. They observed that main root length, total alkaloid and reserpine contents were unaffected by soil moisture.