Chapter 1

Introduction and Review of Literature
Vegetables are the fresh and edible portions such as roots, stems, leaves, fruits or seeds of herbaceous plants. They are important food and contribute minerals, vitamins, and fiber to the diet and are highly beneficial for the maintenance of health and prevention of diseases. Each group contributes to our diet in its own way (Robinson, 1990; Hanif et al., 2006). Vegetables due to their high nutritive value constitute the most important and inexpensive component of a balanced diet and are indispensable for the body. India is the second largest vegetable producer in the world, next only to China with an annual production of 81 million tons from 5.1 million hectares of land (Robinson, 1990; Karanth, 2002; Neeraj and Verma, 2010).

Potato is the world's fourth important food crop after wheat, rice and maize because of its great yield potential and high nutritive value (Raben et al., 1994). It constitutes nearly half of the world's annual output of all root and tuber crops. With an annual global production of about 300 million tonnes, potato is an economically important staple crop in both developed and developing countries. Potatoes are grown in about 140 countries throughout the world and more than a billion people worldwide eat potato. About 328.87 million tonnes of potato are produced in the world over an area of about 19.13 million hectares (Malik and Tufail, 1984; Walker et al., 1999; Pasche et al., 2004; Barthan Roy, 2011-12).

India is ranked 4th in area and is the 3rd largest country in the world in production of potato after China and Russian Federation (Barthan Roy, 2011-12). Potato is produced in an area of 14.00 lakh ha with a production of 250 lakh tonnes and productivity of 17.86 ton per hectare. Potato is grown almost in all states of India. Major potato growing states are Himachal Pradesh, Punjab, Uttar Pradesh, Madhya Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Bihar and Assam. 90% of the potato crop in India is cultivated on Indo-Gangetic plain from October until February-March (PSR 2006; Barthan Roy, 2011-12; Dey and Das, 2015). In India Rajasthan state has a very negligible area under potato cultivation which is restricted to limited pockets like Kota, Dholpur, Bharatpur and Alwar districts (Singh et al., 2007).
Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae. It is an economical food and a source of low cost energy to the human diet (Walker *et al.*, 1999). It is also used for production of high quality starch, alcohol, dextrin, glucose etc. It contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and natural phenols (Malik and Tufail, 1984; Nunn and Qian, 2011). Chlorogenic acid constitutes up to 90% of the potato tuber natural phenols. Other phenolic compounds found in potatoes are 4-O-cafeoylquinic acid (crypto-chlorogenic acid), 5-O-cafeoylquinic (neo-chlorogenic acid), 3,4-dicafeoylquinic and 3,5-dicafeoylquinic acids. A medium-size 150 g potato with the skin provides 27 mg of vitamin C (45% of the Daily Value (DV)), 620 mg of potassium (18% of DV), 0.2 mg vitamin B6 (10% of DV) and trace amounts of thiamin, riboflavin, folate, niacin, magnesium, phosphorus, iron, and zinc. (Raben *et al.*, 1994; Walker *et al.*, 1999; Nunn and Qian, 2011).

Potato is best known for its carbohydrate content (approximately 26 grams in a medium potato). Predominant form of this carbohydrate is starch. A small but significant portion of this starch is resistant to digestion by enzymes in the stomach and small intestine, and so reaches the large intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fiber (Nunn and Qian, 2011). It provides bulk, offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, increases satiety, and possibly even reduces fat storage (Raben *et al.*, 1994). The amount of resistant starch in potatoes depends much on preparation methods. Cooking and then cooling potatoes significantly increases resistant starch. For example, cooked potato starch contains about 7% resistant starch, which increases to about 13% upon cooling (Walker *et al.*, 1999; Fernandes *et al.*, 2005).

This crop is highly susceptible to early blight caused by *Alternaria solani* (Pandey 2007). *Alternaria solani* belongs to the sub-division Deuteromycotina; class Hyphomycetes, family Dematiaceae, with a polycyclic life cycle (Wharton and Kirk, 2007). It reproduces asexually by means of conidia. In the spring, conidia are produced. Multicellular conidia are splashed by water or by wind onto
an uninfected plant. The conidia infect the plant by entering through small wounds, stomata, or direct penetration (Folsom and Bonde, 1925). Infections usually start on older leaves close to the ground. The fungus takes time to grow and eventually forms a lesion. From this lesion, more conidia are created and released. These conidia infect other plants or other parts of the same plant within the same growing season. Every part of the plant can be infected and form lesions. This is especially important when fruit or tubers are infected as they can be used to spread the disease. Disease severity and prevalence are highest when plants are mature (O’Brien and Rich, 1976; Wharton and Kirk, 2007; Yanar, 2011).

In potato, primary damage by *Alternaria solani* is attributed to premature defoliation of potato plants, which results in tuber yield reduction. Foliar symptoms of early blight first appear as small, irregular to circular dark brown spots on the lower (older) leaves. Excessive defoliation may lead to death of the plant and consequent yield loss (Pandey 2007). The pathogen can also attack potato tubers and symptoms are circular to irregular lesions that are slightly sunken and often surrounded by a raised purple to dark brown border and produce a shallow, dry, corky rot (Folsom and Bonde, 1925; O’Brien and Rich, 1976; Wharton and Kirk, 2007). Yield losses up to 79 percent from early blight damage have been reported from India (Pscheidt and Stevenson, 1988, Shtienberg et al., 1990, Franc, 1995, Waals et al., 2004; Pasche et al., 2004, 2005; Pandey, 2007; Yanar, 2011; Dey and Das, 2015).

This excessive economical loss in potato crop can be controlled by applying natural measures like use of plant based herbal formulations. These formulations not only will improve the nutritional quality of crop but well also provide a safe preventive measure to control fungal disease. Hence present chapter deals with incorporation of facts and findings done on control of early blight of potato caused by *Alternaria solani*.

Plant diseases create challenging problems in commercial agriculture and pose real economic threats to both conventional and organic farming systems. Disease management is complicated by the presence of multiple types of pathogens. For any one crop the grower must deal with a variety of fungi, bacteria, viruses and nematodes (Bokshi et al., 2003). This situation is even more
complicated for organic vegetable growers because they usually produce a wide array of vegetable crops and are prohibited from applying conventional synthetic fungicides (Gurjar et al., 2012). The world market continues to be extremely competitive and continues to require that growers supply high-quality disease free produce with an acceptable shelf life. Disease management is therefore a critical consideration in organic vegetable production (Koike et al., 2000; Karanth, 2002; Adhikari et al., 2017).

Man is dependent on plants for almost every need and requirement. Hence, destruction of crop plants due to infection by fungal pathogens has always been an area of prime concern. Scientists all over the world are involved in finding methods of developing techniques for control of plant diseases. Chemical control is the most common and prevalent method of disease control (Masuduzzaman et al., 2008). Synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective (Bokshi et al., 2003, Osman and Al-Rehiayam 2003; Sharma et al., 2008; Anitha and Miruthula, 2014). Mancozeb (0.2%) was found most effective for inhibiting the mycelial growth of Alternaria solani (Choulwar, 1989). The effectiveness of mancozeb in controlling early blight of tomato has also been reported (Singh et al., 2001; Siva et al., 2008).

The negative side of the use of synthetic fungicides is that they are harmful for human and animal health as well as soil. They enter the food chain and cause several deleterious effects on biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification and compaction, thatch accumulation, and diminished resistance to diseases (Shiva et al., 2004; Hada and Sharma, 2014). The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems (Siva et al., 2008, Masuduzzaman et al., 2008, Gurjar et al., 2012; Bektas & Eulgem, 2015; Sonker et al., 2017).

Thus, current thinking about plant and environment protection suggests alternatives to pesticides and use of other strategies in addition to well known disease management methods such as crop rotation, use of resistant cultiva,
planting disease free seeds, biological control etc. (Tuzun & Kloeppep, 1995). Natural plant products are important sources of new agrochemicals for the control of plant diseases (Kagale et al., 2004, Maya and Thippanna, 2013). A search for an environmentally safe and economically viable strategy for the control of diseases has led to an increased use of plant based products in agriculture. Plant product preparations and bio–agents do not leave any toxic residues and therefore can effectively replace synthetic fungicides (Krupinsky et al., 2002, Coelho de Souza et al., 2004; Danish et al., 2011; Hajra et al., 2011; Jabeen et al., 2013; Hada and Sharma, 2014).

Recent trends favour the use of alternative substances derived from natural plant extracts to control pests (Lale and Abdulrahman, 1999; Xan et al., 2003; Islam et al., 2004). The use of natural products for the control of fungal diseases in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment (Cao and Forrer, 2001b; Khair and Haggagg, 2007). These natural products or plant extracts can be exploited either as leads for chemical synthesis of new agrochemicals, or as commercial products in their own right, or as a source of inspiration to biochemists for the development of new bioassays capable of detecting other, structurally simpler, compounds with the same mode of action (Lange et al., 1993; Islam et al., 2004; Yanar, 2011; Jabeen et al., 2013).

As the focus of the world is shifting towards natural products and analogues, the demand of herbal medicine is also increasing and several plants have been screened for activity. Antifungal activity of plant or their extracts as well as essential oil have been studied by several workers (Ballal et al., 2001; Satya et al., 2005; Akinpelu et al., 2006; Buwa et al., 2006; Guleria and Kumar, 2006; Cavaleiro et al., 2006; Tegegne, 2007; Liasu and Ayandele, 2008; Tariq et al., 2008; Bobbarale et al., 2009; Audipudi et al., 2010; Ashraf et al., 2011; Sheikh et al., 2012; Rajamanickam and Sudha, 2013, Hada and Sharma, 2015a; Deepak and Gopinath, 2017).

In the present study herbal formulation from Cassia fistula L. fruit pulp extract in combination with various elicitors like neem, mustard and coconut oil cake and binders like cow dung, guar gum and gum acacia were assayed for their antifungal activity against Alternaria solani.
Cassia fistula (Linn.) belongs to family Fabaceae and Sub–family Caesalpinioideae. It is a very common plant known for its medicinal properties and is semi-wild in nature. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil (Prashanth et al., 2006; Bhalerao et al., 2012). It is commonly known as Amaltas and in English it is popularly called “Indian Laburnum” and has been extensively used in Ayurvedic system of medicine for various ailments. It is deciduous and is found in mixed-monsoon forests throughout greater parts of India, ascending up to 1300 m in outer Himalaya. It is widely used in traditional medicinal system of India (Solecki, 1975; Kirtikar and Basu, 1984; Khare, 2007; Gupta, 2010; Danish et al., 2011).

According to Hartwell (1967-1971), the plants are used in folk remedies for tumors of the abdomen, glands, liver, stomach, throat cancer carcinomata and impostumes of the uterus. Root is useful in fever, heart diseases, retained excretions and biliousness (Nadkarni, 2009). Cassia fistula leaves are crushed to prepare a thick paste and mixed with coconut oil. This paste is applied over the burnt skin twice a day (Patil, 2012). Juice of leaves is used as dressing for ringworm, relieving irritation and relief of dropsical swelling. The pulp of the fruit as well as flowers and seeds are mild purgatives (Mohd. et al., 2011). Ashes from burnt pods mixed with little salt and taken with honey 3- 4 times a day relieves cough. Fruits are used as cathartic and in snake bite (Satyavati and Sharma, 1989; Gupta, 2010; Danish et al., 2011).

The antimicrobial activities of Cassia fistula plant parts have been studied earlier by many scientists (Phongpaichit et al., 2004; Prashanth et al., 2006; Sharma et al., 2006; Abubacker et al., 2008; Hajra et al., 2011; Bhalodia and Shukla, 2011; Bhalodia et al., 2012; Hada and Sharma, 2015a; Hada and Sharma, 2015b). Significant reduction in growth of pathogen like Fusarium oxysporium, Rhizopus stolonifer by ethanolic leaf extract of Cassia fistula has been reported (Hajra et al., 2011). Cassia fistula fruit pulp extract showed antifungal activity against Aspergillus niger, Aspergillus clavatus, Candida albicans (Bhalodia et al., 2012).
Dhawan (1994) has reported that aqueous extract of *Cassia fistula* leaves can be used as a biocontrol agent for *Parthenium hysterophorus*. Nematicidal activity of *Cassia fistula* leaves has been reported by Sharma and Trivedi (1994). Antitussive activity of methanolic extracts has been studied by Bhakta *et al.*, (1998). Methanolic extract of seed showed antitumor activity against Ehrlich Ascites Carcinoma (Gupta and Mazumdar, 2000). Sharma and Basandrai (1999) used *Cassia fistula* plant extract for the management of Karnal bunt of wheat. Samy *et al.*, (1998) and Ali *et al.*, (2004) reported that *Cassia fistula* exhibited antifungal and antibacterial activity against fourteen pathogenic bacteria and six pathogenic fungi.

Antimicrobial screening of plant extracts is usually done with crude alcohol or aqueous extracts prepared either by cold or hot extraction methods. Crude or alcohol extract of several plants have been screened for their possible antimicrobial activities against pathogenic virus, bacteria, fungi and protozoa (Mahmoud, 1999; Digrak *et al.*, 1999; Bowers and Locke, 2000; Eksteen *et al.*, 2001; Hol and Van-veen, 2002; Magama *et al.*, 2003; Gulluce *et al.*, 2003; Afolayan, 2003; Meena, *et al.*, 2003; Phongpaichit *et al.*, 2004; Harlapur *et al.*, 2007; Fawzi, *et al.*, 2009; Shanmugavalli *et al.*, 2009; Pawar, 2011; Shabir *et al.*, 2011; Rosa *et al.*, 2012; Maya and Thippanna, 2013; Anitha and Miruthula, 2014; Hada and Sharma, 2015a). Pretorius *et al.*, (2002) tested crude extracts from thirty nine plant species for their antifungal potential against seven economically important plant pathogenic fungi.

All the active principles present in plants are usually aromatic or saturated organic compounds so they get extracted in ethanol or methanol (Cowan, 1999). Some proteins and glucosides etc. are soluble in water hence antimicrobial assay of antimicrobial principle is usually done with aqueous, 50% alcohol or 100% alcohol extracts.

Mughal *et al.*, (1996) observed that aqueous leaf extracts of *Allium sativum*, *Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternata*, *Alternaria brassicola* and *Myrothecium roridum*. According to Khan *et al.*, (1998) aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosporium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera*
against *Alternaria redicina*. Bajwa *et al*., (2001) assayed the antifungal activity of aqueous extract of *Parthenium hysterophorus*, a herb, against *Drechslera hawaiensis*, *Alternaria alternata* and *Fusarium moniliforme*.


Antimicrobial activity of aqueous leaf extracts of *Ageratum conyzoides Boerhaavia diffusa*, *Dathura stramonium*, *Euphorbia hirta*, *Hypitis suaveolens*, *Jatropha gossypifolia*, *Phyllanthus niruri*, *Prosopis juliflora*, *Solanum nigrum*, *Tridax procumbens* and *Ziziphus jujuba* against *Xanthomonas campestris, Agrobacterium rhizogenes* and *Aspergillus fumigatus* has been reported by Sheikh *et al*., (2012). Antimicrobial potential of crude extract of *Moringa oleifera* and *Allamanda cathartica* against multiple drug resistant clinical pathogens has been studied by Rajamanickam and Sudha (2013). Al Akeel *et al*., (2014) has observed antibacterial activity of crude protein extracts from seeds of six different medical plants (*Foeniculum vulgare, Cucumis sativus, Ammi majus, Allium ascolinicum, Cichorium intybus, Rumex vesicarius*) against standard bacterial strains.

Initial antimicrobial screening with crude extract is followed by screening of extracts prepared in various organic solvents. These extracts are studied to search for various phytochemicals, responsible for antimicrobial activity. Tatli and Akdemir (2005) reported antibacterial potential of methanolic extract of *Turkish Verbascum* spp. against *Candida albicans*, *Cryptococcus neoformans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus* and *Mycobacterium intracellulare*. Saadabi (2007) studied the antimicrobial activity of aqueous, chloroform and methanol extract of *Lawsonia inermis* against six
fungal pathogens (*Epidermophyton floccosum, Microsporum odouinii, Trichophyton rubrum, Trichophyton concentricum, Trichophyton tonsurans* and *Candida albicans*) and four human pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa*).

Jayaraman *et al.*, (2008) studied antimicrobial activity of ethyl acetate, acetone, chloroform and water extract of *Stevia rebaudiana* leaves against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Bacillus subtilis, Aeromonas hydrophila, Vibrio cholerae, Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes* and *Epidermophyton* species. Nguyen *et al.*, (2009) studied antymycotic potential of Cinnamon extract against *R. solani*. Ashraf *et al.*, (2011) reported antimicrobial activity of methanol, chloroform and aqueous extracts of *Origanum vulgare* against nine different gram negative and gram positive bacterial strains and three fungal stains. The bacterial strains were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Micrococcus luteus* (ATCC 9341), *Pseudo-monas aeruginosa* (ATCC 33347), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 19430), *Shigella flexneri* (ATCC 25929), *Salmonella paratyphi* (ATCC 9150) and *Proteus mirabilis* (ATCC 49565) and fungal strains were *Aspergillus flavus, Aspergillus niger* and *Aspergillus pterus*.

Nikolajeva *et al.*, (2012) have reported antimicrobial activity of aqueous and ethanolic extracts of eleven *Bryophyta* species and nine *Marchantiophyta* species collected in Latvia against *Staphylococcus aureus, Escherichia coli* and *Bacillus cereus*. Bhagwat and Datar (2013) observed *in vitro* antifungal activity of extracts of leaves and rinds of *Garcinia indica*, rhizomes of *Curcuma aromatica*, roots of *Glycyrrhiza ghieliae*, leaves of *Nyctanthes arbour-tristis* and seeds of *Vernonia anthelmintica* against *Rhizopus stolonifer, Botrytis cinerea* and *Colletotrichum coccodes*. Otto *et al.*, (2014) have studied antibacterial activity of leaf and root extracts of *Cassia alata* against *Neisseria gonorrhea*. Mishra *et al.*, (2017) have reported *in vitro* antibacterial activity of crude extracts of nine tropical flowering plants (*Anogeissus acuminata, Azadirachta indica, Bauhinia variegata, Boerhaavia diffusa, Punica granatum, Soymida febrifuga, Terminalia chebula, Tinospora cordifolia* and *Tribulus terrestris*) against UTI causing MDR bacteria.
Antifungal activity of petroleum ether, chloroform and acetone and ethanol extracts of *Calendula officinalis* against *A. fumigatus*, *Rhizopus japonicum*, *C. albicans*, *C. tropicalis* etc. has been investigated (Kasiram et al., 2000). Jayaprakash et al., (2001) evaluated turmeric oil for its antifungal activity against *A. flavus*, *A. parasiticus*, *Fusarium moniliforme* and *Penicillium digitatum*. Chloroform, ethyl acetate and aqueous extracts of husk of *Cocos nucifera* showed antibacterial activity against several bacteria (Srinivas et al., 2003).

Obafemi et al., (2006) reported that hexane, ethyl acetate and methanol extracts of *Tithonia diversifolia* exhibit antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus* and others. Aqil and Ahmad (2007) reported antibacterial properties of ethyl acetate, acetone and methanol extract of traditionally used Indian medicinal plants. Bobbarala et al., (2009) reported antifungal activity of some medicinal plants against phytopathogenic fungus *Aspergillus niger*. Goyal et al., (2011) reported antibacterial activity of methanol, ethanol, ethyl acetate and aqueous leaf extract of *Ocimum sanctum* against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

Medicinal plants have generated the interest of man for therapeutic values chiefly because of the presence of secondary metabolites. It is obvious that plants have their own built in defense mechanism against infection by almost all microorganisms. Upon recognizing the invading pathogen, they synthesize several secondary metabolites such as phenolics, phytoalexins and lignins and try to ward off the pathogen (Bleve-zacho et al., 1990; Mithyadevi et al., 2007; Anitha and Miruthula, 2014; Khatoon et al., 2017).

The antimicrobial properties of plant extracts therefore, are a result of presence of secondary metabolites such as alkaloids, phenols, flavanoids, terpenoids, essential oils etc. (Harborne, 1984). These secondary metabolites do not play any vital role in building and maintaining processes of plant cells. Several workers have reported antimicrobial activity of these secondary metabolites (Kishore et al., 2000; Sartoratto et al., 2004; Solis et al., 2004; Deng and Nicholson, 2005; Satya et al., 2005; Chapagain et al., 2007; Bakar et al., 2009; Benn et al., 2009; Danish et al., 2011; Bektas & Eulgem, 2015).
Ellof (1998) reported that tannins, saponins polypeptides and reducing sugars are soluble in water whereas terpenoids, flavonoids, alkaloids and fatty acids are soluble in organic solvents. Similar findings have been reported by several workers (Scalbert, 1991; Mendoza et al., 1997; Zhang and Lewis, 1997). Tannins and reducing sugars are soluble in both water as well as organic solvents but their solubility is more in organic solvents as compared to water. Harborne (1984), Kokate et al., (1990) suggested that extraction of secondary metabolites from plant material by hot extraction with petroleum ether separates sterols, waxes and fatty acids leaving behind residue containing the defatted plant materials. Subsequently extraction of this residue with benzene separates out sterols and flavonoids. Terpenoids and flavonoids get extracted with chloroform. The last solvents i.e. alcohol removes alkaloids, flavonoids, polyphenols, tannins and reducing sugar from residue. Finally extraction with water yields remaining water-soluble metabolites such as anthocyanin, starch, tannins, saponins, reducing sugar and polypeptides (Scalbert, 1991; Zhang and Lewis, 1997). All the active principles present in plants are saturated organic compounds so they get extracted in ethanol or methanol (Cowan, 1999).

Flavonoids have existed since one billion years and survived in vascular plants throughout evolution indicating their importance in nature. They are low molecular weight, polyphenolic compounds available in practically all dietary plants (Ren et al., 2003). They represent a widespread group of water-soluble phenolic derivatives, which are mostly brightly coloured. Over 4000 structurally unique flavonoids have been identified in plants. The association between plant flavonoids and various animal species and wide range of biological activities of flavonoids has been reported (Ebadi, 2002). Flavonoids are known to be synthesized by plants in response to microbial infection and their inhibitory activity is due to formation of complexes with extracellular and soluble proteins and bacterial cell wall and disruption of microbial membranes (Tim Batchelder, 2004). Polyphenols, phenolic acids and flavonoids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory actions (Mattila and Hellstrom, 2007; Galeotti et al., 2008; Sharma et al., 2009; Shabir et al., 2011).
Phenols and polyphenol group of compounds consist of thousands of diverse molecules with heterogenous structure and common feature of having one or more phenol ring. They are synthesized in plants by shikimic acid pathway. The site and numbers of hydroxyl groups on the phenol ring is related to their toxicity to microorganisms hence, increased hydroxylation results in increased toxicity (Geissman, 1963). Several workers have reported that phenolic compounds such as caffeic acid, cinnamic acid, catechol, pyrogallol, eugenol, coumarins etc. show antimicrobial activity against virus, bacteria and fungi (Taguri et al., 2004; Saify et al., 2005; Satya et al., 2005; Galeotti et al., 2008).

Alkaloids are heterocyclic nitrogen compounds synthesized by decarboxylation of amino acids. Cinchona alkaloids present in the bark of Cinchona sp. have quinine as their main constituent, which is known since 1630 for its antimalarial activity. Diterpenoid alkaloids isolated from the family Ranunculaceae are commonly found to have antimicrobial properties (Omulokoli et al., 1997).

Tannin refers to polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, known as astringency. Tannins are divided in two groups: hydrolysable and condensed tannins. Condensed tannins, which are generally known as proanthocyanidins are derived from flavonoid monomers (Tim Bachelder, 2004). Their molecular weight ranges from 500 to 3000 (Haslam, 1996) and they are found in almost every plant part: bark, wood, leaves, fruits and roots (Scalbert, 1991). Tannins work by stimulation of phagocytic cells, host-mediated tumor activity and a range of anti-infective actions as well as the ability to form complexes with proteins (Haslam, 1996). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelop, transport proteins etc. Scalbert (1991) reported the antimicrobial properties of tannins. According to his studies, tannins can be toxic to filamentous fungi; yeasts and bacteria. Condensed tannins have been reported to bind with cell walls of ruminal bacteria, preventing their growth and protease activity (Jones et al., 1994). Several workers have reported antimicrobial activity of tannins (Hori et al., 2006; Reddy et al., 2007).
Saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but lower marine animals and some bacteria are also known to produce these compounds (Riguera, 1997; Yoshiki et al., 1998). Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid in nature. A large number of the biological effects of saponins have been ascribed to their action on membranes. In fact, their specific ability to form pores in membranes has contributed to their common use in physiological research (El Izzi et al., 1992; Authi et al., 1988; Choi et al., 2001; Menin et al., 2001; Plock et al., 2001). Saponins have been described for hypocholesterolaemic potential (Potter et al., 1993; Matsuura, 2001), anticarcinogenic activity (Mimaki et al., 1998; Podolak et al., 1998), antifungal potential (Delmas et al., 2000; Wang et al., 2000), antiviral and antiprotozoanal potential (Newbold et al., 1997; Apers et al., 2000).

The fragrance of plant is due to presence of quinta essentia or essential oil fractions. These oils are highly enriched secondary metabolites that are based on isoprene units. They are also called as terpenes. Their general chemical structure is C_{10}H_{16} and they occur as diterpenes, triterpenes and tetraterpenes (C_{20}, C_{30} and C_{40}) as well as hemiterpenes (C_{5}) and sesquiterpenes (C_{15}). When the compounds contain additional elements usually oxygen, they are termed as terpenoids (Cowan, 1999).

Antimicrobial terpenoid were isolated from *Pterocarpus indicus* by Ragasa et al., (2005). Ten sesquiterpenes and six diterpenes were isolated and screened for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Rhizoctonia solani* etc. (Solis et al., 2004). Several workers have reported antimicrobial activity of terpenes or essential oil (Tepe et al., 2004; Mamtha et al., 2004; Saroglou et al., 2005; Al Dabbas et al., 2005; Garcia Vallejo et al., 2006; Kuiate et al., 2006). Plant extracts also exhibit trypanocidal, leishmanicidal and antimalarial activity.
The biological and molecular action of secondary metabolites induces various morphological and cytological changes in microorganisms. These changes can be studied at microscopic as well as macroscopic level. Macroscopic changes include change in colony colour, shape, size etc. Changes in cell number, cell size, cell shape, number of reproductive structure can be observed at microscopic level. Effect of extract on cytomorphology i.e. cell size; cell shape and cell number of different organisms has been studied by several workers (Burt and Reinders, 2003; de Leon et al., 2005; Zhang et al., 2006; Goel and Sharma, 2013; Hada and Sharma, 2018).

Burt and Reinders (2003) reported that oregano essential oil brings about extensive morphological changes in treated cells. The cell structures appeared to be empty of contents and remains were flaccid while control cells were found to be whole. Zeylastral and demethylzeylastral, two phenolic compounds isolated from the roots of Maytenus blepharodes (Celastraceae), showed inhibition of synthesis of DNA, RNA, protein and cell wall (de Leon et al., 2005). Complete inhibition in the incorporation of the N-aceylyl-D-I-14C glucosamine suggests that the phenolic compounds compromise the cell wall synthesis and/or cytoplasmic membrane. Zhang et al., (2006) isolated steroid saponin from Tribulus terrestris L. and these steroid saponins showed significant in vitro and in vivo antifungal activity, weakening the virulence of Candida albicans and killing fungi through destroying the cell membrane. Goel and Sharma, (2013) reported that leaf and inflorescence extracts of Euphorbia pulcherrima showed significant cytomorphological changes in Aspergillus fumigatus.

There are several methods available to assay antimicrobial sensitivity. Poison food technique (Grover and Moore, 1962) and Disc or Agar well diffusion methods (Collee et al., 1996) are commonly used to determine antimicrobial sensitivity test. Sensitivity of microbes against plant extracts by Poisoned Food Technique has been studied by several workers (Kumar et al., 2006; Pattnaik et al., 2012; Sohbat et al., 2013; Singh et al., 2013; Al-Rehman et al., 2013; Deelip et al., 2013; Mehta and Sharma, 2013, Parveen and Sharma, 2014; Hada
and Sharma, 2015a; Hada and Sharma, 2016; Hada and Sharma, 2018). This method measures percent inhibition of the tested microbes. The activity of extract is always compared with that of the control.

Agar well diffusion method depends on the inhibition of bacterial growth as an indication of activity, magnitude of which is measured as a function of the diameter of inhibition zone. The activity of extract is always compared with that of the currently used antibiotic in parallel line assay. Sensitivity of microbes against plant extracts by agar well method has been studied by several workers (Shittu et al., 2006; Erturk et al., 2006; Natarajan et al., 2007; Okore et al., 2007; Abere et al., 2007; Bhalodia and Shukla, 2011). Antimicrobial activity by disc diffusion method has also been studied by several workers (Satya et al., 2005; Khosravi and Behzadi 2006; Erturk et al., 2006; Ayandele and Abebiyi, 2007; Usman and Osuji, 2007; Usman et al., 2007; Vadlapudi and Naidu, 2009; Derbalah et al., 2011; Asuquo et al., 2017).

Although the diffusion method is commonly used in preliminary susceptibility testing but it is not always an accurate method to assay antimicrobial activity because there is a high degree of interference with this method, arising from drug diffusion problems. A more generally accurate method of assessment is the broth dilution technique (Collee et al., 1996). Therefore the broth dilution method was used to determine antimicrobial activity measured as MIC (Jabeen et al., 2013; Hada and Sharma, 2015a).

In the diffusion methods there is the limited diffusion of the less polar active compound in solid media, which shows the lack of inhibition zone while in the broth dilution method the compounds in solution come in direct contact with the organisms (Rios et al., 1988; Silva et al., 1996). Okore et al., (2007) assayed anticandidal activity of crude aqueous pod extract of *Lecaniodiscus cupanoides* by broth dilution technique. Antimicrobial sensitivity by broth dilution technique has been reported by several workers (Wilson et al., 2005; Obafemi et al., 2006; Usman et al., 2007; Yanar, 2011; Jabeen et al., 2013).
Plants are naturally gifted at the synthesis of medicinal compounds. The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value (Colegate and Molyneux, 1993; Maya and Thippanna, 2013; Anitha and Miruthula, 2014). A classic example is aspirin, which was initially discovered as salicylic acid in willow bark and leaves, another noted example is taxol, recently proven to be effective against breast and ovarian cancers, which was initially discovered in bark of yew trees (Donehower and Rowinsky, 1993). The use of medicinal plants (herbs) has a long history throughout the world and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of numerous countries (Hostettmann et al., 1995; Shabir et al., 2011; Rosa et al., 2012; Hada and Sharma, 2014).

Plant extracts have been reported to act as elicitors or induce defense mechanisms in plants. Addition of organic matter improves crop yield mainly by enhancing soil fertility (Bhalodia and Shukla, 2011). Considerable improvements in soil structure, water retention capacity and aeration in different types of soils have been observed following the addition of green manures, farm yard manures and other organic matter. Another additional advantage of using organic matter is the activation of many beneficial microbes antagonistic to soil borne pathogens, leading to disease suppression (Vidyasekaran, 1988; Bobbarale et al., 2009; Ashraf et al., 2011).

In plant biology elicitors are extrinsic, or foreign, molecules often associated with diseases or synergistic organisms and plant pests. Molecules of elicitor can attach to specific receptor proteins located on plant cell membranes (Sheikh et al., 2012). The molecular pattern of elicitors is recognized by receptors and trigger intracellular defence signalling via the Octadecanoid pathway. The response results in the enhanced synthesis of metabolites which decrease damage and increase resistance to pest, disease or environmental stress (Bektas & Eulgem, 2015).
Oil cake is one of the natural organic fertilizers with high nitrogen content, which is the residues of neem seeds, mustard, peanut seeds, sesame, coconut etc. after oil extraction process of the processing plant. (Hada and Sharma, 2016). Antifungal activity of various elicitors has been studied by several workers (Rajan and Singh, 1973; Arora and Kaur, 1999; Babu et al., 2000; Rao et al., 2003; Kimaru et al., 2004; Bektas & Eulgem, 2015; Hada and Sharma, 2016).

Kimaru et al., (2004) investigate effect of Neem Kernel Cake Powder (NKCP) on development of tomato Fusarium wilt. Farmers in India use neem leaves to protect stored grain from insects. Neem (Azadirachta indica) is considered to be one of the most valuable trees of the 21st century for its great potential in pest management, effective source of environmentally powerful natural pesticides, environmental protection and medicine. The waste by-product remaining after the oil extraction processes is neem cake. Neem is considered devoid of toxicity, as tested also by the old traditional use. Neem oil cake has been successfully utilized as livestock feed for growing goats (Rao et al., 2003).

Binder is a material which holds or draws other materials together to form a cohesive whole mechanically, chemically, or as an adhesive. Generally materials labeled as binders in various proportions or uses can have their roles reversed with what they are binding. Antifungal activity of various binders has been studied by several workers (Duke, 1985; Dobelis, 1986; Matsuzaki et al., 1998; Naskar & Ray, 2003; Sravani et al., 2014; Zandraa et al., 2015; Hada and Sharma, 2016).

Guar gum, also known as guaran, is primarily the ground endosperm of guar beans. The guar seeds are dehusked, milled and screened to get the guar gum. It is produced as a free-flowing, off-white powder. The color of guar gum powder is whitish and yellowish having slight odor (Sravani et al., 2014; Zandraa et al., 2015). Acacia gum has long been used in everyday applications and in traditional medicine. The material is used by Egyptians as glue and as a pain reliever base. Arabic physicians with the gum treated a wide variety of ailments
Presently, it is used widely in the cooking industry to give body and texture to processed food products and in the pharmaceutical industry as a demulcent. It is also used to stabilize emulsions. The bark fibers are used to make cordage (Duke, 1985).

Cattle rearing in India has been a tradition and intimately limited to agricultural economy. Different products used widely in number of Ayurvedic formulations are obtained from cow milk, ghee, curd, urine, and dung. In Indian sub-continental farming cow dung is traditionally used as organic fertilizer for centuries. The addition of cow dung increases the mineral status of soil, also increases resistance of plant against pests and diseases; increases plant growth and other beneficial activities such as sulphur oxidation and phosphorous solubilization. The Composition of cow dung is around 80% water and supports a matrix of undigested plant material that is rich in nutrients, micro-organisms, and their byproducts (Matsuzaki et al., 1998; Naskar & Ray, 2003; Hada and Sharma, 2016).

The world is gradually turning to herbal formulations which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects and are readily available at affordable prices (Kirtikar and Basu, 1984; Sharma et al., 2008). However, add a note of caution stating that plant remedies are effective and without side-effects, provided they are selected properly and taken under proper medical supervision. The active component, most often a secondary metabolite, varies in quality and quantity for a given plant species growing in different locations (Prajapati et al., 2004; Purohit and Vyas, 2004).

Herbs and spices, such as basil and clove possess antimicrobial properties and have been used to protect food from spoilage since time immemorial (Arora and Kaur, 1999). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Medicinal value of plants depends on these inherent substances that produce a definite physiological action on the human body (Edeogo et al., 2005). In recent
times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems (Dutta, 1973; Vishwanathan and Basavaraju, 2010).

The herbal formulations made from plants have been used against several phytopathogens, the formulations are used on the basis of nature of plant disease as well as nature of pathogen. There are several methods like soil amendment, root dipping, seed dressing, foliar spray etc. for management of various crops. Organic matters are also important for plants and help to overcome the plants against pathogen attack. Organic matter influences physical characters of soil such as pore size, aeration, temperature, water retention capacity etc. which help in better solubilization of minerals. They also provide nutrients on decomposition (Schlumbaum et al., 1986; Mandelbaum and Hadar, 1990; Hoitink et al, 1997).

There are several traditional agricultural practices followed by farmers to control plant diseases. Probably the oldest document was Kautilya’s Arthasastra, which reported the use of organic materials to control the crop disorders. Formulation is a cheap, environmentally safe fungicide made by combining plant extracts and organic materials to control plant diseases. Many of such techniques of traditional agriculture require validation, such as use of organic materials (cow dung, oil cakes etc.) for control of plant diseases (Nene, 2003; Gomase et al., 2011).

Antifungal activity of herbal formulations has been studied by several workers (Dutta, 1973; Rajan and Singh, 1973; Lootsma and Scholte, 1996 Davis et al., 1996; Ivanyuk et al., 1996; Matsuzaki et al., 1998; Arora and Kaur, 1999; Gamliel et al., 2000; Razia Akbar, 2000; Edeogo et al., 2005; Hamid et al., 2008; Vishwanathan and Basavaraju, 2010; Akila et al., 2011; Gomase et al., 2011; Hada and Sharma, 2016).

Matsuzaki et al., (1998) reported that soil with cow manure amendments is best treatment for reducing the severity of the disease and improving the final tubers yield of potato. Similar findings were stated by Davis et al., 1996; Ivanyuk et al., 1996. Reduction in the stem infection has also been observed when oats preceded potato as a green manure crop (Lootsma and Scholte, 1996). Solarizing
soils plus use of suitable organic materials have also been reported to actuate a chain reaction of chemical and microbial degradation, which enhance toxicity against soil flora and fauna, especially soil borne plant pathogens. These probably contributed to the higher nutrient contents, which could be found with organic manure amendment (Gamliel et al., 2000; Matsuzaki et al., 1998).

Rajan and Singh, (1973) reported that soil amendments with castor, groundnut, sesame, margosa, coconut oil cakes and sawdust with and without incorporation of urea were significant against Pythium in ginger soft rot. Hamid et al., (2008) used cow manure and soil solarisation treatment for effective suppression of potato disease caused by R. solani and subsequent improvement of the final tuber yield. Use of cow dung for smearing the cuttings of fig before planting is mentioned in Dara Shikoh’s documents (Razia Akbar, 2000).

Commercial viability of any herbal formulation or plant extract depends on its ability to maintain stability at varying physical conditions. Antimicrobial activity of plant extracts depends on chemical nature of compounds present in them. Various physical factors such as pH, temperature, and exposure to sunlight may bring about a change in chemical nature of these compounds. Several workers have studied the effect of various physical factors on efficacy of extract (Jeong et al., 2004; Lee et al., 2004; Hada and Sharma, 2017b).


*In vitro* studies are always followed by *In vivo* studies to confirm the results in natural conditions for successful transfer to technology. Several workers have conducted *In vivo* studies for study of antifungal activity of plant extracts using pot experiments (Nidiry, 1999; Shailbala and Tripathi, 2004; El-Mougy and Abdel-Kader, 2009; Ganie et al., 2013; Hada and Sharma, 2017a; Adhikari et al., 2017).
Al-Mughrabi *et al.*, (2006) conducted field trials against foliar and tuber disease of potato by using three types of organic material including thermal compost, static wood chips and vermin castings. Infection of *R. solani* afflicted the normal growth and yield of potato tubers. However treatment with extract was been helpful in resuming normal yield (Khair and Haggag, 2007). Seed dipping method and foliar spray were used for the study of preventive effect of herbal formulation have been reported by several workers (Jan *et al.*, 2003; Ganie *et al.*, 2013; Hussein and Hamideldin, 2014; Hada and Sharma, 2017a).

Although antimicrobial activity of *Cassia fistula* L. has been studied by some workers but not much work has been done to develop herbal formulation from *Cassia fistula* L. fruit pulp extract in combination with elicitors and binders for control of early blight of potato caused by *Alternaria solani*. Similarly not many reports are available on antimicrobial activity of elicitors and binders.

Hence, antifungal activity of crude, partially purified fractions by soxhlet and column fractions of *Cassia fistula* fruit pulp against *Alternaria solani* was also done by using standard methods described in chapter 2. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of selected plant extract has been also determined during the study.

Elicitors like neem, mustard and coconut oil cake and binders like cow dung, guar gum and gum *Acacia* individually and in combination with plant extract have been also assayed for antifungal activity against *Alternaria solani*.

Preliminary screening of the active fractions of the extract has been done to determine the phytochemicals present. The separation or purification of these phytochemicals has been done by different chromatographic techniques such as TLC, column chromatography and Gas chromatography (GC). Mass spectrometry (MS) study was done to characterization of the compounds present in purified GC fractions.

The mode of action of inhibition by studying the effect of plant extract on growth and reproduction of test pathogen has also been made, effect of various physical factors on efficacy of extract as well as herbal formulation and in vivo study of preventive effect of herbal formulation against potato crop has also done.