Research Article

Ecofriendly management of late blight of potato caused by *Phytophthora infestans* (L)

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ABSTRACT

Potato is an important vegetable crop which is cultivated all over the world. Potato crop is highly affected by *Phytophthora infestans* at low temperature and high humidity. In severe case whole field converted into burnt blacken field. Many chemical fungicides are used to control this pathogen but these fungicides also affect soil and water microflora and environment become affected. Present study focused to avoid these adversely affects on environments and used green materials like plant extracts to control phytopathogenic fungi *Phytophthora infestans* that cause late blight of potato. Some plant extracts showed highly effective activity while some of them were not effective at al.

Objectives: Application of ecofriendly, toxin free method to control phytopathogenic fungi- *Phtyophthora infestans*. Some plants are collected from the field of Meerut and Hardwar region and powdered after drying at 60°C overnight in the oven and powdered green materials were subjected to convenient process: soxhlet extraction unit (Virot *et al*., 2007). Solvent prepared at different concentrations and discs applied on potato leaves after inoculating pathogen on leaves (Heatley, 1944). *Terminalia* sp and *Psoralia* sp showed encouraging result while some plant extracts were not effective.Plant extracts are fully safe to the environment and not toxic to anyone.

Keywords: Ecofriendly, *Phytophthora infestans,* Botanicals, phytopathogenic fungi.

INTRODUCTION

Phytophthora infestans is highly devastitating disease and cause economic loss. To control this late blight pathogen many contact and systemic fungicides are used. Systemic fungicides are effective than contact fungicides. (Rani et al., 2007a, 2007b, 2009; Wong et al., 2001). Most of the fungicides are restricted due to the harmful effects of the fungicides on the environment by causing water and soil pollution and hence directly animals and humans are affected (Harris et al., 2001; Komarek et al., 2010) but these chemicals are highly on demand to restrict their use in the field to kill phytopathogens and introducing new management strategies. Decades of research on application of eco-friendly methods for plant disease management have not yielded the desired result of complementing or totally replacing the pesticides. However, the no. of fungicides that are available to regulate plant diseases is dwindling due to swift development of resistance by pathogens. Plant pathologists are therefore left with no choice but to re-evaluate the available viable alternatives such as biocontrol, inorganic salts and natural plant products (botanicals), which are ecofriendly, biodegradable and less expensive (Zaker, 2016) Although biocontrol is rarely used for foliar diseases, numerous organisms capable of antagonizing fruit and leaf pathogens reported in recent years. Formerly, little attention was given to biocontrol of foliar pathogens, principally because the foliar microflora was known to consist of relatively few organisms whose populations fluctuate dramatically according to environmental conditions. The foliage growth provides surpass contigency for phytopathogen growth rather than organisms found in the soil surrounding plants (Fry, 1982; Rani et al., 2007). Biocontrol of an airborne foliar disease is the use of the antagonistic bacteria *Pseudomonas cepacia* and *B. subtilis* to control *Monilia* pod rot in cocoa (Falconí et al., 2002). Certain microorganisms in phyllosphere are antagonistic to *P.infestans, Sporobolomyces* spp., *Acetenobacter* spp. and isolates of *Pseudomonas* spp. and *Bacillus* spp. were reported by Ramos et al., (1993) and Sanchez et al., (1998). Another important alternative of fungicides application that involves the use of inorganic salts to regulate plant disease. Hill et al*.,* (1998) while working with wide range of treatments such as Ca chelates, phosphates and pectin against late blight opined that we might be able to use environmentally safe chemicals to check late blight. (Bhat et al., 2007; Anju Rani et al., 2016). Bicarbonate salts control gummy stem blight (*Didymella bryoniae*) and leaf blight (*Alternaria cucumeriania*) in muskmelon grown in green house (Ziv and Zitter, 1992). Effectiveness of plant extracts against *P.infestans* demonstrated by many workers (Latten 1994; Rohner et al., 2004). Blaeser et al., (1998) announced that *Potentilla erecta* and *Salvia officinalis* were highly

effective against *P. infestans* on solanaceous plants (tomato) under greenhouse conditions. Locally available plant species such as *Annona squamosa*, *Thevetia peruviana*, *Pongamia glabra*, neem, *Acorus calamus*, *Psoralea corylifolia* and *Terminalia* spp. have also been reported to be effective against several plant pathogens (Ashrafuzzaman et al., 1990; Khilare and Gangawane, 1997; Shenoi et al., 1998; Mungkornasawakul et al., 2002; Kiran and Raveesha, 2005; Rani Anju et al., 2015; Liang *et al*., 2012). Neem products has been commercialized to minimize the effects of many phyto-pathogenic fungi (Locke, 1995; Rani *et al*., 2006). Objective of my disquisition is to use ecofriendly fungicides or biofungicides to control phytopathogen *Phytophthora infestans*.

Materials and methods

Twenty seven plant species *viz*., *Terminalia bellerica* - seed, *Tamarindus indica* - leaf, *Annona squamosa* - leaf, *Pongamia pinnata* - leaf, *Ricinus communis* - seed, *Phyllanthus asperlatus* - seed, *Cassia tora* - leaf, *Ocimum basilicum* - leaf, *Brassica* sp. - leaf, *Datura metel* - leaf, *Ficus* sp. - leaf, *Acorus calamus* - seed, *Mentha arvensis* - leaf, *Ficus religiosa* - leaf, *Withania somnifera* - stem, *Asparagus racemosus* - leaf, *Alstonia scholaris* - leaf, *Thevetia pereviana* - leaf, *Cassia fistula* - pod, *Cymbopogan flexuosus* - leaf, *Chrysanthemum* - flower, *Cannabis sativa* - leaf, *Citrus sp*. - leaf, *Nictenthus urbertsistis* - flower, *Azadirachta indica* - seed, *Psoralea corylifolia* - seed, *Cassia angustifolia* - leaf were collected from Meerut and Haridwar area, brought to laboratory, shade dried and kept in oven at 60 ^{0}C overnight for drying. Dried plant parts were powdered in a mixer. Powdered plant parts were then subjected to hot solvent extraction (soxhlet extraction unit) methanol, acetone and hexane (Virot et al., 2007). Extraction was carried out for 18 h at specified boiling temperature of 69^0, 65^0 and 56 ^{0} C for hexane, methanol and acetone respectively. The extracts obtained in flask were subjected to vacuum flask evaporator to retrieve solvents. The extract was collected in preweighed labeled plastic vials and the original weight of the extract was determined. Mother extracts were prepared in acetone to get the final concentration of 100 %. Each vials containing the extracts were properly labeled and parafilm was wrapped around the cap of the vial to prevent evaporation and stored in refrigerator till further use. In order to see the efficacy of plant extracts against late blight, extracts of all plant species were tested by detached leaf method 5,000 ppm and 10,000 ppm. Zoosporangial suspension containing 6x10^4 zoospores / ml was prepared and counted by haemosytometer. Fungal spore suspension was prepared by taking 1ml spore suspension was gently mixed with 1ml of water (1:1 ratio). This suspension of spores were sprayed on to the potato leaflets placed in the tray. Five replications were maintained for each treatment and three leaflets constituted one replication. To obtain 10,000-ppm concentration, calculated amount of the mother extract was incorporated into 25 ml sterile distilled water using an auto pipette. Accordingly other concentrations were prepared. For proper mixing of the extract with water, 500 μl of tween 20 was added to the solution. After spraying sporangial suspension, plant extracts solution was sprayed on to the leaves till run off and allowed to shade dry. Observations on lesion size and number of zoosporangia in different treatments were taken. Sporangia presence was confirmed by microscopic studies. (Plate 1).

The greatest width and length of individual lesions was measured after 5 days and based on this, lesion area was calculated using the following formula

$$LA = \frac{\Pi ab}{4}$$

where, a and b are the length and width of the lesion respectively (Singh and Bhattacharya, 1995). Sporulation capacity (number of zoosporangia/cm^2) was also measured after 5 days of incubation. The sporulating lesions of all the leaflets were cut and dipped in vials containing 10 ml of 10% ethyl alcohol. The vials were stored at 4°C.

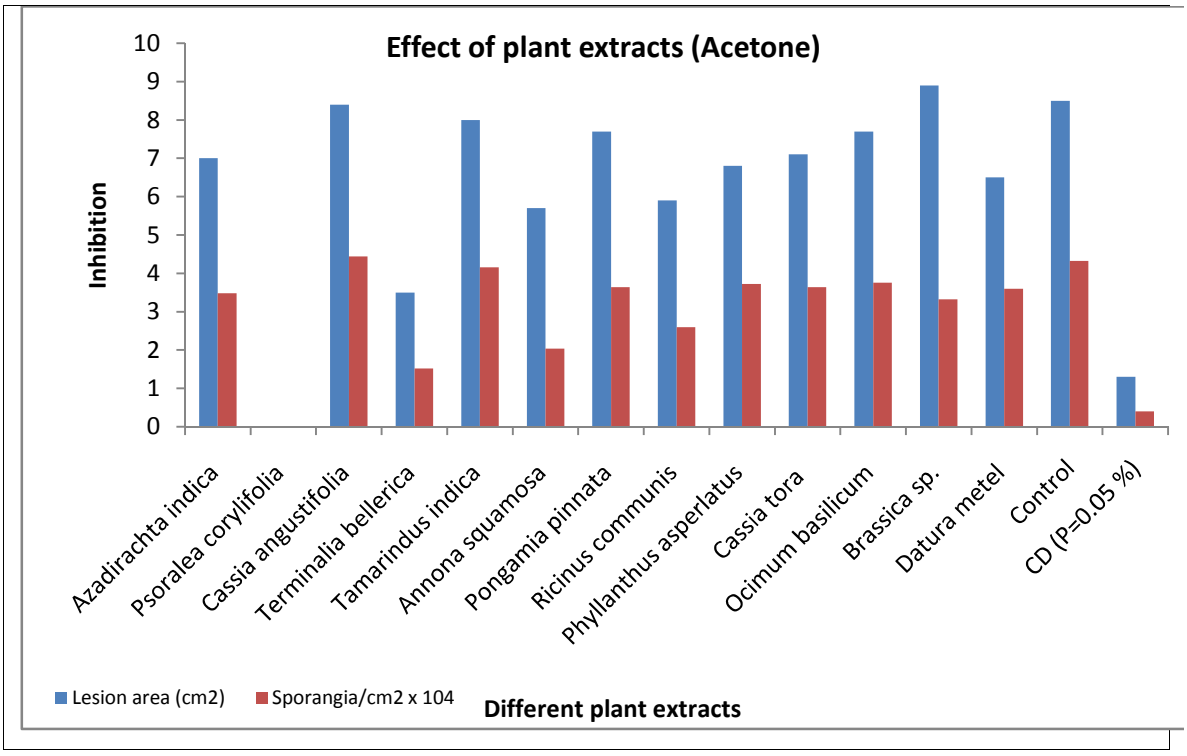
Result

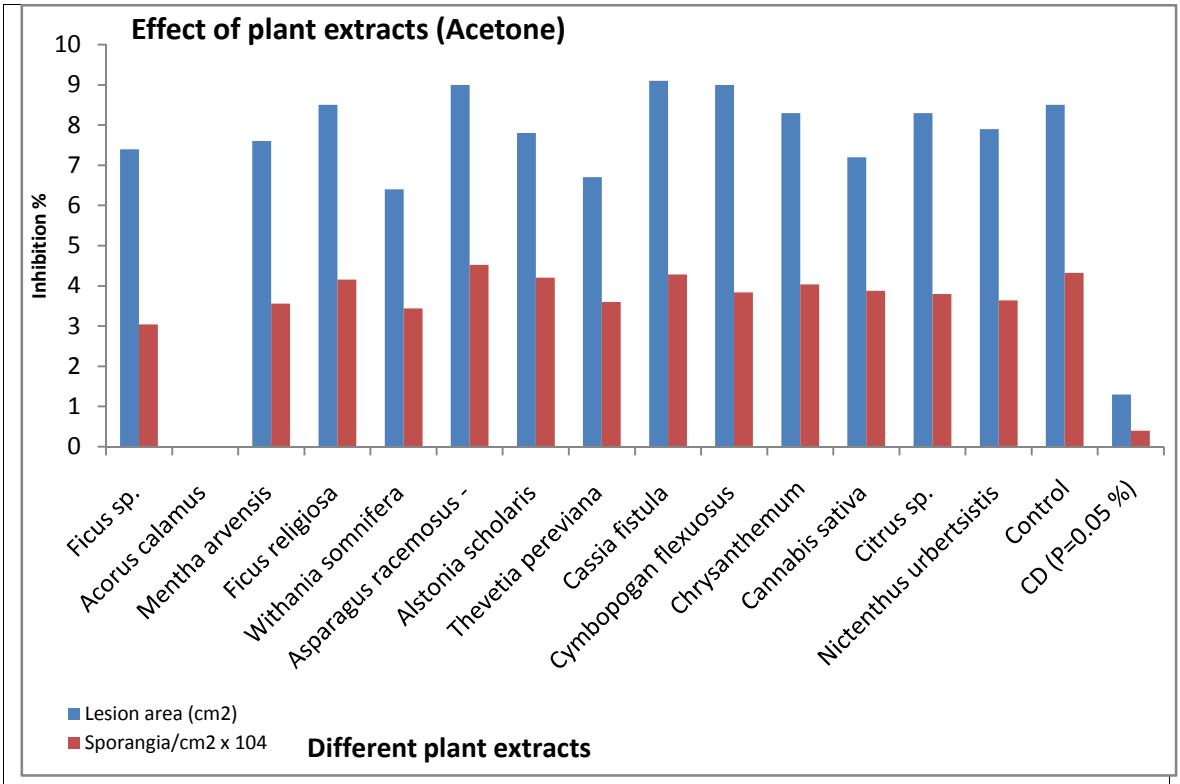
All the 27 plant species were tested by detached leaf method at 10,000 ppm. Among the acetone extracts *Psoralea corylifolia* and *Acorus calamus* completely inhibited *P.infestans* and were significantly superior to other plant species (Table 1 and Plate 1). *Terminalia bellerica* showed encouraging effect. *Asparagus racemosus* treatment had a stimulatory effect which produced 4.52 x 10 ^{4} sporangia / cm^2 as against 4.32 x 10 ^{4} sporangia / cm^2 in untreated control. Rest of the extracts showed varied effect. Among the methanol extracts, *Terminalia bellerica* and *Acorus calamus* completely inhibited the lesion size. *Psoralia corylifolia* extract was also highly effective which produced only 0.5 cm^2 lesion size as against 8.6 cm^2 in untreated control. Rest of the extracts showed little effect. Sporangial production varied in different plant extracts with highest (4.5x10 ^{4} sporangia / cm^2) production recorded in *Ficus religiosa* and the least (0.1 x 10 ^{4} sporangia / cm^2) in *Psoralia corylifolia*. Among the hexane extracts, *Psoralia corylifolia*, *Terminalia bellerica*, and *Acorus calamus* were highly effective and were at par. Extracts of other plant species were either less effective or non-inhibitory. Similarly, *P.corylifolia*, *T.bellerica* and *A.calamus* extracts also reduced sporangial production. Sporangial production in these three extracts were 0.16, 0.52 and 0.24 x 10 ^{4} /cm^2 respectively as against 4.28 x 10 ^{4} /cm^2 in untreated control.

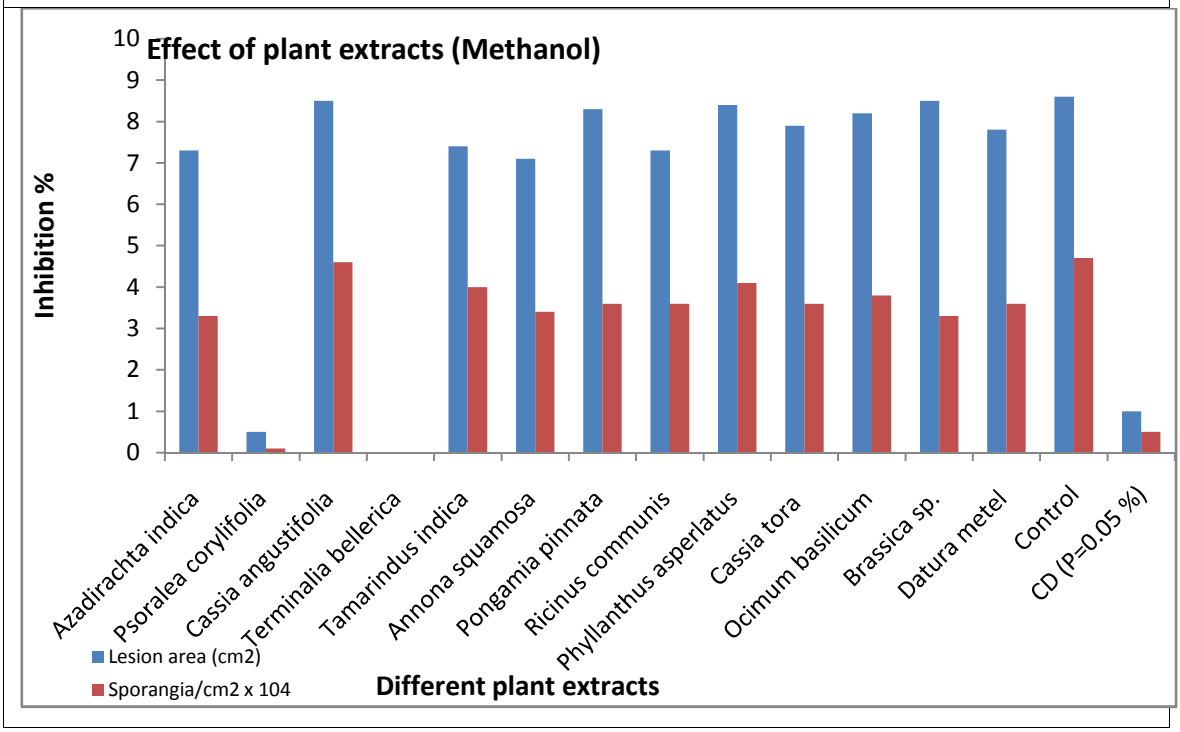
Discussion and conclusion

Under the present study, acetone, methanol and hexane extracts of twenty-seven plant species tested

in vitro and *in vivo* against *P.infestans*. Results revealed that acetone, methanol and hexane extracts of *Acorus calamus*, *Terminalia bellerica* completely inhibited the pathogen. *Thevetia pereviana*, *Brassica* sp., *Annona squamosa* and *Pongamia pinnata* showed inhibitory effect on *P.infestans in vitro* but were found ineffective under detached leaf test. Their efficacy against *P.infestans* is not known but they have shown inhibitory effect against large of fungal plant pathogens. Acetone extracts of *Psoralea corylifolia* also showed similar result. *Withania somnifera*, *Cannabis sativa*, *Thevetia pereviana*, *Brassica* sp., *Annona squamosa* and *Pongamia pinnata* showed > 50 % zoospore germination inhibition. Zoospores were comparatively more sensitive to plant extracts than mycelium. In detached leaf test, acetone extract of *Psoralea corylifolia* and acetone and methanol extract of *Acorus calamus* and methanol extract of *Terminalia bellerica* completely inhibited late blight development. Efficacy of plant extracts against *P.infestans* has been demonstrated by several workers (Latten. 1994; Neuhoff *et al*., 2002; Rohner *et al*., 2004; Anju Rani et al., 2015). Sardsud *et al*., (1994) reported that *A calamus* extract at 1 % inhibited the growth of *Botryodiplodia theobromae* completely. Mungkornasawakul, (2000) demonstrated the antifungal activity of crude dichloromethane extract of *A. calamus* rhizomes using *Cladosporium cladosporioides* as test fungi. Later, Mungkornasawakul *et al*., (2002) tested dry and powdered rhizome extracts of *A. calamus* against *Alternaria* and *Fusarium* spp. (Crusifer wilt) as well as *Botrytis* spp. (rose gray mold rot) and *Septoria* spp. (chrysanthemum leaf spot) and result of these indicated that all the examined pathogens were sensitive to *A.calamus* extract. Phongpaichit *et al*., (2005) reported that *A. calamus* inhibited the growth of *Trichophyton rubrum, Microsporum gypseum* and *Penicillium marneffei*. The extracts of *A. calamus* have been found to possess anti bacterial activity also (Grosvenor *et al*., 1995; McGaw *et al*., 2002; Rani *et al*., 2003). Shenoi *et al.,* (1998) evaluated several medicinal plants against *A. alternata* on tobacco *in vitro* and reported that *Thevetia peruviana, Lawsonia inermis, Leucas aspera* and *Pongamia glabra* were highly effective. Fungicides are popularly used to control the phytopathogens but also leave harmful aftereffects and afftect many soil and water microorganisms. (Wightwick & Allinson, 2007;Anju Rani et al., 2017). Plant extracts are not only effective against phytopathogens but also effective against human pathogens. (Maneesha Singh et al., 2016).The seeds of *P. corylifolia* used as an ancient Hindu remedy for psoriasis, leucoderma, and inflammatory diseases of the skin (Latha *et al*., 2000). The rhizomes utilized extensively by the Chinese, Indians and American Indians (Motley, 1994). The rhizome considered to possess anti-spasmodic, anthelmintic and carminative properties and also used for treatment of chronic diarrhea, epilepsy, mental ailments, intermittent fevers, dysentery, tumors and bronchial catarrh. It is listed as a fish toxin, antibacterial agent, insecticide, and antifungal agent (Anonymous, 1975). Hence, plant extracts are fully safe and ecofriendly in nature. There are no harmful effects on host as well as soil and water microorganisms.




Effect of plant extracts (Acetone)
Inhibition %
10
9
8
7
6
5
4
3
2
1
0
Ficus sp.
Acorus calamus
Mentha arvensis
Ficus religiosa
Withania somnifera
Asparagus racemosus -
Alstonia scholaris
Thevetia pereviana
Cassia fistula
Cymbopogan flexuosus
Chrysanthemum
Cannabis sativa
Citrus sp.
Nicitenthus urbertsistis
Control
CD (P=0.05 %)
Lesion area (cm2)
Sporangia/cm2 x 104
Different plant extracts


Effect of plant extracts (Methanol)
Inhibition %
10
9
8
7
6
5
4
3
2
1
0
Azadirachta indica
Psoralea corylifolia
Cassia angustifolia
Terminalia bellerica
Tamarindus indica
Annona squamosa
Pongamia pinnata
Ricinus communis
Phyllanthus asperlatus
Cassia tora
Ocimum basilicum
Brassica sp.
Datura metel
Control
CD (P=0.05 %)
Lesion area (cm2)
Sporangia/cm2 x 104
Different plant extracts

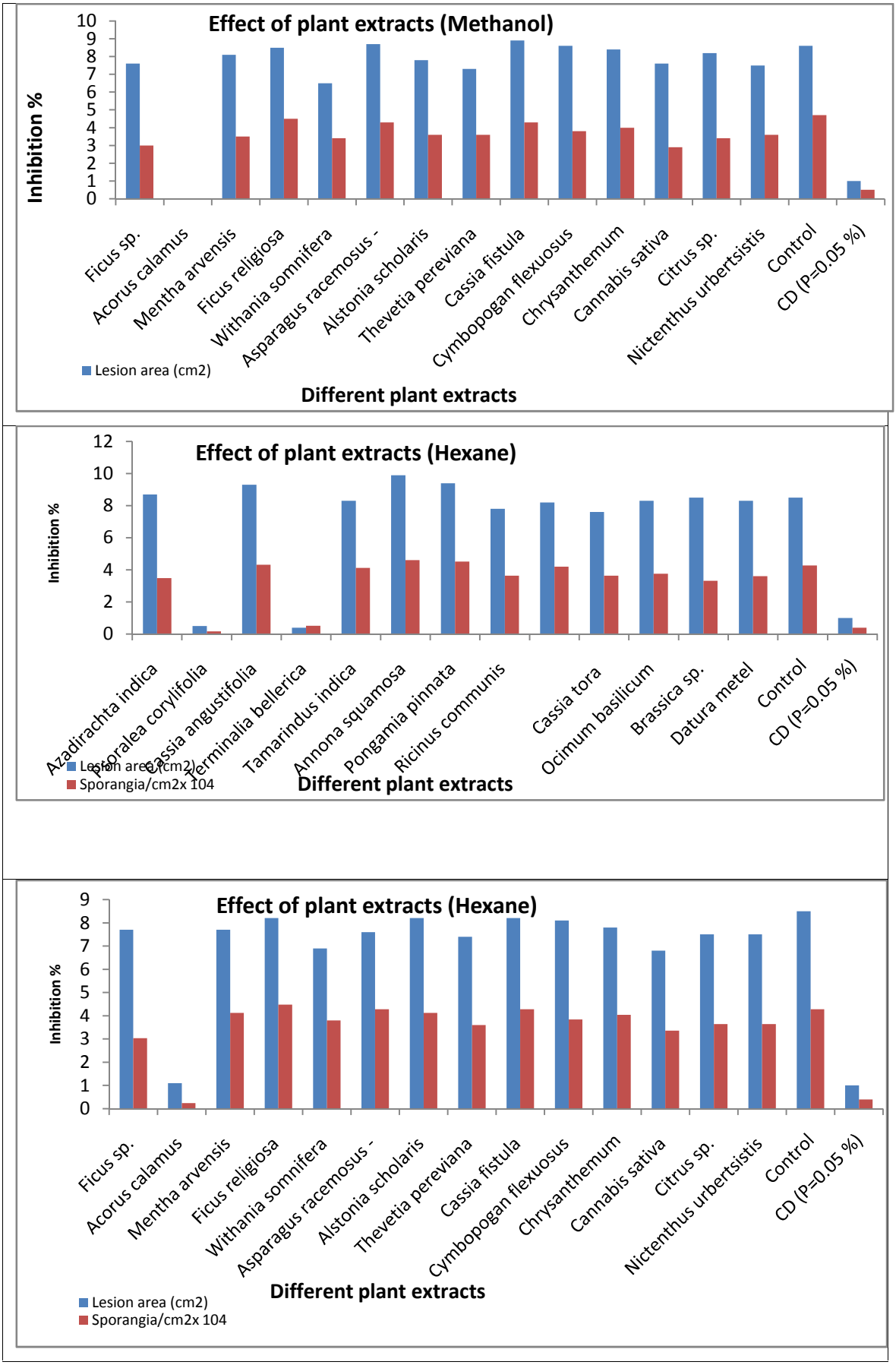


Fig. 1: Effect of different botanicals against *P. infestans* by detach leaf method

Fig. 2: Effect of different botanicals on detached potato leaves.

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