International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 30-08-2021, Accepted: 15-09-2021, Published: 30-09-2021
Volume 6, Issue 5, 2021, Page No. 913-920

Endorsement of antifungal properties of medicinal plants extract in post-harvest diseases management of *Colletotrichum capsici*

Uma Chaurasiya, Sapan Patel*
School of studies in Botany, Jiwaji University, Gwalior, Madhya Pradesh, India

Abstract
Extensive application of pesticides especially fungicides for crop production resulted in adverse consequences such as resistance development in pathogens and environmental pollutions. An alternative approaches in agriculture are requisited for environmental sustainability. Hence, an investigation was conducted with medicinal plants like *Adhatoda vasica* and *Acalypha indica* to exploration antifungal compounds to inhibit the growth of post -harvest pathogen as *Colletotrichum capsici* in chilli fruit. Ethanolnic and methanolnic of extract of leaf and stem of *Adhatoda vasica* and *Acalypha indica* by using soxhlet to study the effect on chilli *Colletotrichum capsici*. The pathogen, *C. capsici* was isolated from the infected part of fruit surface of chilli. Aqueous, 70 % ethanolic and methanolic extract of *Adhatoda vasica* and *Acalypha indica* were screened, against *C. capsici*. The colony diameter of control and extract treated fungus was measured with the help of scale to determine percentage growth inhibition of fungus. Conidia germination inhibition carried out at Minimum inhibitory concentration (MIC) and Medium inhibitory concentration (IC50) of ethanolic and methanolic extracts. The leaf and stem extracts of *Acalypha indica* extracted in the ethanolic solvent expressed higher antifungal activity than aqueous and methanolic extract. Conidial germination percentage inhibition was reported 90.38 % at MIC concentration of *A. vasica* leaf extract of ethanolic followed by ethanolic stem extract of *A. vasica*, Methanolic leaf and stem extract of *A. indica*, respectively. Appressorium formation in *C. capsici* was inhibited completely at MIC concentration of ethanolic leaf extract of *A. vasica*, but the delay was observed by methanolic extract of leaf and stem.

Keywords: medicinal plant, anthracnose disease, chilli, plant pathogens, crude extract of the plant

Introduction

Chilli is an important commercial vegetable crop of the Solanaceae family which grown all over the world. Chilli has pungency due to the presence of alkaloid “capsaicin” in its pericarp and fruit placenta that give mild to intense spice. Capsaicin is used against gastrointestinal disorder such as dyspepsia, loss of appetite, gastroesophageal reflux disease, gastric ulcer. Metabolites of chilli reduce gastroesophageal reflux disease (GERD) symptoms, inhibits gastrointestinal pathogens, reduce ulceration and cancers (Gogoi *et al*., 2018) [11]. Vitamins, minerals, and oil are rich in Chilli due to this property; it keeps a special place in food recipes as well as in pharmaceutical (Akarsh *et al*., 2016) [2]. India is the largest producer of chilli and share the global production of 47% with 0.7–0.8 million hectares area followed by China 7%, Thailand 5.9% and Pakistan 3.5%. Telangana, Karnataka, Madhya Pradesh and Orissa are the major state of chilli producing in India. The area cultivated with chilli is about 23 thousand hectares with (7 hundred thousand acres) producing 1.8Million. Madhya Pradesh is the third rank, contribute 7.24% of the national chilli harvest. 8821.05 metric toneschilli were lost after post-harvest in Madhya Pradesh in 2018 (FAO, 2018). Production and maintaince of chilli crop is difficult due to biotic and abiotic factors which influence the yield of crops (Kumar *et al*., 2018) [16]. Abiotic factors include adverse climatic condition (temperature and humidity), irrigation, nutrient supply while biotic factors includes insects, mites, nematodes, rodents, slugs and snails, birds, weeds, viruses, bacteria and fungi which drastically reduce the crop yield and among these biotic factors pathogenic attack more specifically the fungi pathogen is most determining factor for chilli production. Plants are affected at developmental stages as well as during postharvest conditions from the fungus that lead to quality-related issues such as varied organoleptic characteristics, nutritional values and reduced shelf life (Mesta *et al*., 2007) [19]. *Colletotrichum capsici* cause anthracnose in chilli which is common agronomical issue linked with chilli production in the tropics and subtropics area. Several fungicides such as mancozeb, captan, bavistin, thiram, copper oxychloride, cosan, benlate, andziram and others fungicides are used to control *C. capsici* infection. Residues of these fungicides remain in the harvested fruit that lead to environmental pollution and toxic effects on human health (Salam *et al*., 2018) [26]. An extensive use of fungicide caused pathogen resistance development toward fungicides. As aesthetically and economically, chilli and its products are highly important and hence, there is a urge of chilli plant protection via sustainable approaches such application of plant curde extract or uses of biocontrol microorganisms (Mishra *et al*., 2019) [20]. *Bacillus* sp., produce 55 kDa protein that reduces anthracnose disease development in chilli (Srikhong *et al*., 2018) [32]. Moreover, *Pseudomonas aeruginosa* has been found to produce biosurfactant (Rhamnolipid) that reported inhibiting *C. capsici* growth *in-vitro* and after detaching fruit from the plant (Lahkar *et al*., 2018) [17]. Jaihan *et al*., (2018) [14] also evaluated that mycelium extract of *Ophicordyceps sobolifera* and isolated bioactive constitute adenosine and cordytopolone reduce the disease severity of anthracnose disease of chilli via the pot experiment. Nduagu

et al., (2008) [22] studied the leaf, stem and root bark aqueous extract of *Annona senegatensis*, *Azadirachta indica*, *Chromolaena odorata*, *Citrus limon*,*Cochlospermum planchonii*, *Hymenocardia acida*, *Ocimum gratissium*, *Psidium guajava*, *Ricinus communis*, *Tephrosia vogelii* and *Vernonia amygdalina* to observe inhibition of colony diameter and sporulation of *C. capsici*. But no report is available for the effect of methanolic, ethanolic plant extract against *C. capsici* on mycelium growth, conidia germination and appressorium formation. Thus, though literature is available on the reduction of disease by bacteria, fungi and plant extract but the effect of bacteria, fungi and plant extract on appressorium are not provided. Therefore, the present study aimed to investigate the effect of aqueous, methanolic and ethanolic extract on appressorium formation. The study ultimately aims to use of plant extracts as biocides in place of fungicides to prevent the economic loss due to fungal plant pathogens.

Material and Methods

Isolation and Identification of *Colletotrichum capsici*:

The Pathogen was isolated from the infected chilli fruit (*Capsicum annuum*) area using the standard method (Agostini *et al*., 1992) [1]. The fungus was identified based on microscopic character, taxonomic key, and description (Smith and Black 1990; Sutton, 1980) [31, 33].

Collection of Plant Material:

Adhatoda vasica and *Acalypha indica* stems and leaves were collected from the campus of Jiwaji University, Gwalior, Madhya Pradesh, India (Latitude: 26.20 and Longitude: 78.14) in December 2017.

Preparation of Extract

Collected leaves and stems were weighed and washed by tap water 4–5 times, followed by distilled water. After washing, leaves and stems were air-dried under shade for three weeks and again weighed to determine the moisture content of the sample after that it was grinded into a grinder to made powder. Thirty grams of powder was extracted in soxhlet by using 70 % methanol, 70 % ethanol and distilled water, respectively. Extraction procedure include 0.030 Kg of extract powder was filled in soxhlet thimble and mixed with 0.250 L of solvent followed eight soxhletcycle. The extract was concentrated and dried in Lyophilizer at -55 ^{0}C temperature and 1.0 torr pressure (BioEra -55 ^{0}C model clout). After dried, the crude extract was weighed to determine the yield percentage of extract and store in sterilized vials at 4 ^{0}C.

Crude extract yield was calculated by using the following formula:

$$Yield\ (\%) = (W1 \times 100)/W2$$

W1 is the weight of the dry crude extract, and W2 is the initial weight of dry plant material packed in the Soxhlet.

Antifungal Activity of Extracts

The antifungal activity of methanolic, ethanolic and aqueous extracts of selected plant leaves and stems were evaluated according to food poison technique (Liu *et al*., 2017) [18]. Total 500 ppm volume has taken in 1, 2, 3, 4 and 5 g L^{-1} concentrations of plant extracts and dissolved in 5 % DMSO (SRL), was poured into the center of Petri plate (90 mm) containing 9.50 ml potato carrot agar and kept the plate at room temperature for the absorption of the extract in the medium. Carmel (Carbendazim 12 % + Mancozeb 63 %) was used as positive control and DMSO as a negative control. By using cork borer, 4 mm mycelial disc was placed on the center of each plate and incubated at 27 ^{0}C±1 in the incubator. Radial growth from the center was measured at the interval of 1st, 3rd, and 5th d of the incubation period. The following formula measured the percentage of growth inhibition.

$$\text{Inhibition } \% = \frac{(C-T)}{C} \times 100$$

Where C is the Diameter of a Fungal Colony in Control.
T is the Diameter of a Fungal Colony in the Treatment.
Minimal inhibitory concentration (MIC) and $IC_{50,}$ concentration inhibited more than 50 % growth of fungus, was also be determined by using the food poison method.
The Speed Index Mycelial Growth (SIMG) was observed by using the last day, and the previous day of observing the diameter growth value of fungus expressed in mm per day (Fardin and Young, 2015) [10].

$$SIMG = \frac{\sum(CD - PD)}{N}$$

Where CD: Current average Diameter; PD: Previous average Diameter; N: Number of days after inoculation.

Inhibition of Conidial Germination: Conidial germination was observed by a slightly modified method of DeCorato *et al*., (2017). 20 µl volume of conidial suspension (1×10^5 spore ml^{-1}) was transferred into 100 µl potato dextrose broth (PDB) containing the different concentrations of 880 µl extract in each vial prepared in 5 % DMSO incubated at 27 ± 1 ^{0}C for 48 h incubation. 10 µl culture aliquot was placed on the slide containing lactophenol cotton blue and then covered with a coverslip and was observed under the microscope using a micrometer. One hundred conidia were observed from each replicate. The experiment of each extract and control was estimated using the following formula:-

$$\text{Growth inhibition } = \frac{GC - GT}{GC} \times 100$$

GC is the Germination in Control, and GT is the Germination in the Treatment.

Quantitative Estimation of Flavonoid: Flavonoid estimation was done with a slight modification method described by Sagbo *et al*., (2017) [24]. 0.003 g crude extract was dissolved in 0.00125 L of distilled water. Then, 75 ppm of 5 % sodium nitrite solution ($NaNO_{2,}$ Titan biotech) was added into it. After five min, 150 ppm of 10 % Aluminum chloride ($AlCl_3$) (Titan biotech) was poured into tubes and kept for six min. 500 ppm of 1 M sodium hydroxide (NaOH, Rankem) was added into it and the final volume was maintained up to 0.005 L by adding distilled water and mixed well. The solution was inoculated for 30 min, and further, the absorbance of the solution was measured at 510 nm with Systronics Controller-Based Spectrophotometer 169 (Gujarat, India). The total flavonoid content was calculated by using the following the formula: T = C × V/m,

where T is the Total Flavonoid content; V is the volume of the extract (g) used in the assay, C is the quercetin equivalent (g L^{-1}), and m is the weight (g) of the crude extract used in the assay. Values were expressed as quercetin equivalent per gram of dry plant extract (g Qe/g).

Total Phenolic Content: Total phenolic content of extract was estimated (Folin-Ciocalteu method) with some modifications by Yeoh and Ali (2017). Briefly, 500 ppm extract was mixed with 0.006 L of dH_2O and 0.0015 L of 10 % Folin-Ciocalteu reagent. Then incubated at room temperature for 4 min, followed by 0.0015 L of 7 % sodium carbonate and 0.0019 L of dH_2O were added. The solution was vortexed, then incubated at 37 ^{0}C for 2 h. 500 ppm of dH_2O blank was replaced with 500 ppm extract in blanck. The absorbance was measured against a blank at 765 nm using spectrophotometer. Thereafter, gallic acid (0.05–0.5 g L^{-1}) standard curve equation was used to calculate sample concentration as ggallic acid equivalents per Kg of dried weight sample (g GAE/Kgd.w.). The total phenolic contents were calculated using following formula (Alara *et al*., 2020).

$$TPC = \frac{V * C}{m}$$

Where C is the sample concentration obtained from the calibration curve (g L^{-1}), V is the volume (L) of the solvent used for the extraction, and m represents the weight (g) of the dried sample used.

Data analysis: The experiment was conducted twice in a triplecate. The data obtained from the experiment and analyzed as the mean standard error of the mean.

Results and Discussion

Moisture content and extract yield of *A. vasica* and *A. indica*: *A. indica* leaf and stem possessed higher moisture content than *A. vasica* (Table 1). Aqueous extract yield of *A. indica* was found highly effective than ethanolic and methanolic extract while *A. vasica* yielded higher ethanolic stem extract than the aqueous extract of *A. vasica*. The extraction yield of *A. vasica* found similar to *A. indica* extraction quantity. Results of leaf extract yield were reported similar to (Ishnava *et al.*, 2012) that extract yield is obtained high in aqueous solvent compare to methanol solvent.

Table 1: Shows moisture content percentage and plant crude extract of different in solvent

	Acalypha indica		*Adhatoda vasica*	
	Leaf	**Stem**	**Leaf**	**Stem**
Moisture content %	77.99	71.15	39.00	55.64
Aqueous crude extract (g)	52.70	10.00	32.26	25.00
Ethanolic crude extract (g)	33.00	8.73	5.98	30.10
Methanolic crude extract (g)	22.10	8.86	29.60	11.40

Inhibitory effect of plant extract against *C. capsici*:
The inhibitory effect of *A. vasica* and *A. indica* crude extract were tested against *C. capsici* at varying concentration of 1, 2, 3, 4 and 5 g L^{-1} for fungal growth inhibition. Ethanol extract of *A. indica* was found more effective than *A. vasica*. Fungal colony growth was inhibited from 1 g L^{-1}in leaf and stem ethanolic extract of *A. indica* (Fig. 1A & B) whereas leaf ethanolic extract of *A. vasica* inhibited from 2 g L^{-1} (Fig. 2A). Minimum mycelium growth was 0.50 mm/24h (Fig. 3B) and 94% colony growth inhibition (Fig. 1B) observed at 5 g L^{-1}concentration of ethanolic *A. Indica* stem extract followed by 0.52 mm/24h (Fig. 4A) fungal growth and 93 % growth inhibition (Fig. 2A) at 5 g L^{-1} concentration of ethanolic *A. vasica* leaf extract. Highest growth inhibition was observed in ethanolic leaf and stem extract of both plants when the fungus was treated with 2 g L^{-1} concentration of extract,but maximum fungal growth was inhibited from 4 g L^{-1} extract concentration (Fig. 1 & 2). Sakthi *et al*., (2011) [25] observed a similar result that ethanolic leaf extracts strongly inhibited colony growth of *Candida albicans*, *Candida glabrata*, and *Aspergillus flavus* compared to ethyl acetate leaf extract. Whereas Bapat *et al*., (2016) [4] studied ethanol extract of *A. vasica* as antifungal activity and was not found any positive result against *Sclerotium rolfsii*.
Methanolic leaf extract of *A. indica* expressed 82 % colony growth inhibition (Fig. 1A) of a fungus with 1.79 mm/24h SIMG value (Fig. 3A) at 5 g L^{-1}concentration, whereas methanolic stem extract of *A. indica* exhibited 72 % growth inhibition (Fig. 1B) with 2.61 mm/24h SIMG value (Fig. 3B). Methanolic leaf and stem extract of *A. vasica* showed a similar effect on growth inhibition 73 % and 74 % (Fig. 2A & B) with 2.73 and 2.54 mm/24h SIMG values (Fig. 4A & B). Akarsh *et al*., (2016) [2] also studied the effect of methanolic leaf extract of *A. vasica* on *C. capsici* and *F. oxysporum* f.sp. *zingiberi* and found that extract inhibited 50 % radial growth. However, the extract effect on *C. capsici* was least compare to the present study. Radwan *et al*., (2014) [23] reported that isolated compounds from the methanolic extract of *Myristica fragrans* fruit were not expressed significantly growth inhibition of *Colletotrichum* sp. In our study, Leaf aqueous extract of *A. indica* (Fig. 1A) was not expressed growth inhibition of fungus whereas stem extract of *A. indica* inhibited 21 % at 5 g L^{-1}concentration (Fig. 1B). While *A. vasica* leaf and stem extract inhibited 18 % and 16 % growth (Fig. 2A & B). The results are supported with those of Ishnava *et al*., (2012) [13] who studied the effect of aqueous extract of *A. vasica* against *Alternaria* sp., *Aspergillus parasi*, *Aspergillus nidulans*, *Trichoderma harzianum* and *Aspergillus flavus* and reported that aqueous extract of *A. vasica* was inhibited *Aspergillus flavus* growth rest of fungi.

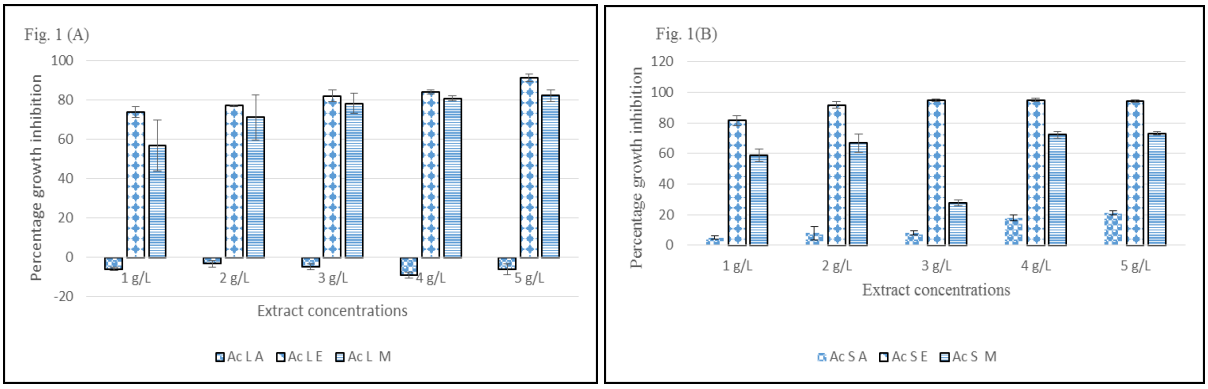

Fig 1: Depict percentage diameter inhibition of *C. capsici* by the effect of *A. indica.* Ac = *Acalypha indica*, *L*= leaf, *S* = stem, *A* = Aqueous, *E*= Ethanol, *M*= methanol.Data are expressed as mean standard error of the mean.

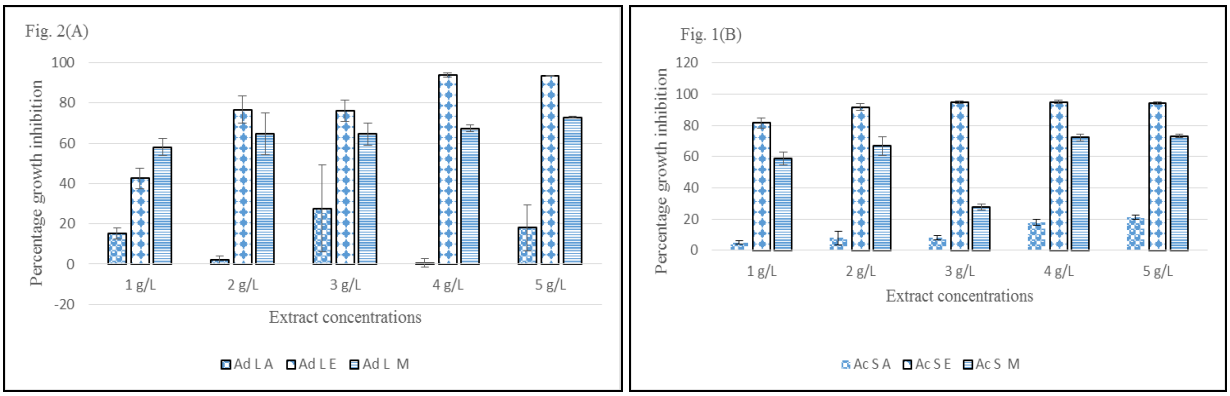

Fig 2: Depict percentage diameter inhibition of *C. capsici* by the effect of *A. vasica.* Ad= *Adhatoda vasica L*= leaf, *S* = stem, *A* = Aqueous, *E*= Ethanol, *M*= Methanol. Data are expressed as mean standard error of the mean.

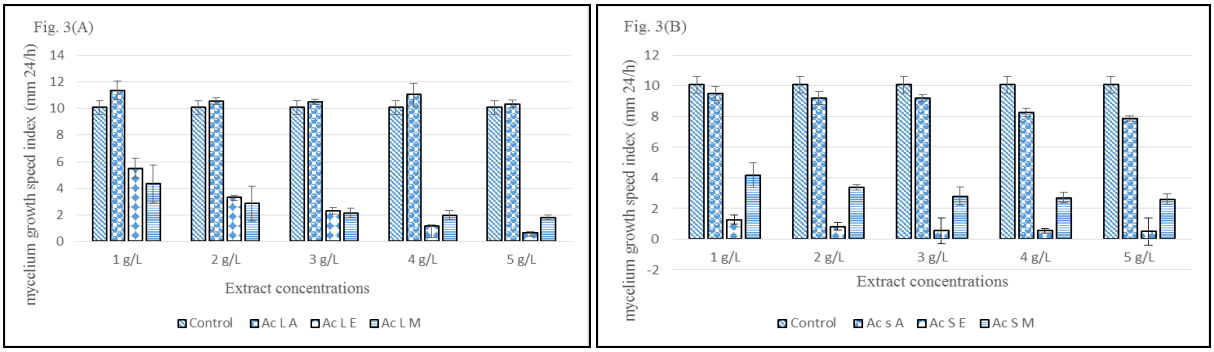

Fig 3: Shows the effect of *A. indica* Leaf (3A) and stem (3B) on mycelium growth speed index (mm 24/h) of *C. capsici*. Ac = *Acalypha indica*, *L*= leaf, *S* = stem, *A* = Aqueous, *E*= ethanol, *M*= methanol. Data are expressed as mean standard error of the mean.

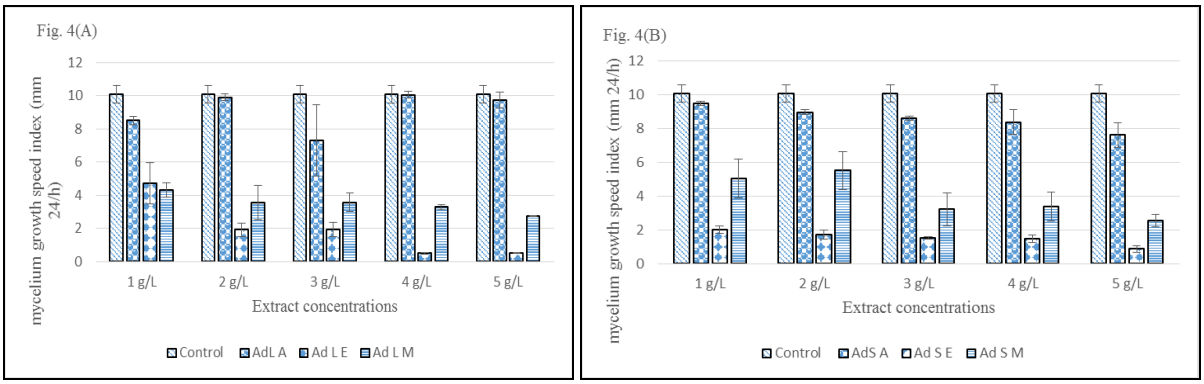

Fig 4: Showsthe effect of *A. vasica* leaf (4A) and stem (4B) on mycelium growth speed index (mm 24/h) of *C. capsici.* Ad = *Adhatoda vasica*, *L*=leaf, *S* =stem, *A* =Aqueous, *E* =ethanol, *M* =methanol. Data are expressed as mean standard error of the mean.

Inhibition of conidial germination: IC_{50} and MIC of *A. vasica* and *A. indica* were tested against conidial germination of *C. capsici* presented in Table 3. Ethanol extracts of plant expressed good conidial germination. The

highest conidial germination was observed at 90 ± 0.38 % by the leaf extract of *A. vasica* at MIC concentration. Lowest conidial germination 36.53 ± 4.98 % was inhibited at IC_{50} concentration of ethanolic leaf extract of *A. indica*. Whereas, the extract of plant parts was found to inhibit conidial germination less than 50 % when conidia were treated with the methanolic extract. Bhutia *et al*., (2016) [5] reported that 0.3 % *Zingiber officinale* rhizome and 0.5 % *Polyanthia longifolia* leaf extract inhibit 68 % conidia germination of *Colletotrichum musae*.

Table 2: Shows Minimum inhibitory concentration (MIC) and Medium (IC_{50}) inhibitory concentration of *A. indica* and *A. Vasica* against *C. capsici* in g/L

Treatments	*Adhatoda vasica*		*Acalypha indica*	
	MIC	**IC_{50}**	**MIC**	**IC_{50}**
A. L. Ex	0	0	0	0
E. L. Ex	4	1.5	5	0.5
M. L. Ex	0	1.5	0	1
A.S. Ex	0	0	0	0
E. S. Ex	5	0.5	2	0.5
M.S. Ex	0	2.5	0	1

L = leaf, S = stem, A = Aqueous, E = ethanol, M =methanol, Ex = Extract.

Table 3: Shows effect of plant extracts on conidia germination percentage inhibition

Treatments	*Adhatoda vasica*		*Acalypha indica*	
	MIC	**IC_{50}**	**MIC**	**IC_{50}**
A. L. Ex	0	0	0	0
E. L. Ex	90.38 ± 1.22	72 ± 6.43	87.5 ± 0.93	36.53 ± 4.98
M. L. Ex	0	52.88±1.36	0	49 ± 0.49
A.S. Ex	0	0	0	0
E.S. Ex	83.65± 2.1	59.61± 1.24	75.96 ± 1.5	39.42 ± 2.99
M.S. Ex	0	46.15 ±7.74	0	47 ±5.4

L = leaf, S = stem, E = ethanol, M =methanol, Ex = Extract. Data are expressed as mean standard error of the mean.

Appressorium is a melanized specialized cell of fungus *Colletotrichum* sp., which is required for infection to plant host cell walls through penetration. Appressoria was not formed from conidia after the treatment of ethanolic leaf (Fig. 5B) and stem (Fig. 5D & E) extract of *A. vasica* but the minimum number of conidia germinated to form only germ tube. Whereas, the bulb-like structure was formed when conidia were treated with methanolic leaf extract of *A. vasica* (Fig. 5C). Appressoria was formed in methanolic stem extract of *A. vasica* (Fig. 5F) but it delays the conidial germination because ofthe very slow growth of appressoria and germ tube than control. Conidia were germinated and form appressoria with germ tube at 0.5 g L^{-1} concentration of ethanolic leaf extract of *A. indica* (Fig. 6A) but at 5 g L^{-1} (Fig. 6B) concentration, conidia was completely inhibited. Methanolic leaf (Fig. 6C) and stem (Fig. 6F) extract of *A. indica* was also responsible for the delay of conidia germination.

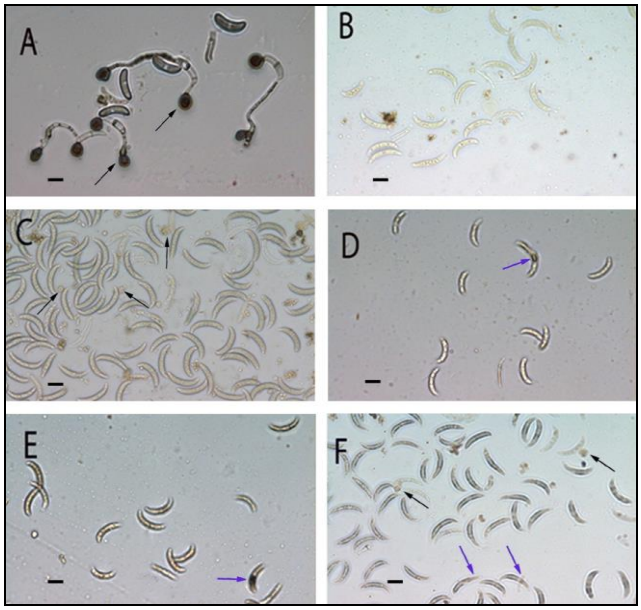

Fig 5: shows the effect of *A.vasica* on conidial germination after 48 hours (A) control. (B) Ethanol leaf extract (5 g L^{-1}) on conidia germination. (C) Methanol leaf extract on conidia germination. (D) Ethanol stem extract (0.5 g L^{-1}) on conidia germination. (E) Ethanol stem extract (5 g L^{-1}) on conidia germination. (F) methanolic stem extract on conidia germination. Black arrow indicated appressoria formation and blue arrow indicate germ tube formation.

Conidia germination was inhibited dueto the incorporation of phytochemicals into the cell membrane and caused the loss of stability and integrity of the cell membrane (Singh *et al*., 2016) [30]. Mitogen activated protein kinase (MAPK) regulated the function of cell wall integrity and control osmostasis during high osmolarity stress of *Saccharomyces cerevisiae* (Herskowitz, 1995) [12]. Mitogen activated protein kinase kinase (MPAKK) STE11 family gene was identified as regulation in the formation of appressorium (Buyu, 2018) [6].

Formation of appressorium regulated by cell cycle in *Colletotrichum* sp. In which microtubules interact with cell polarity determinants in the conidia, when conidia were treated with benomyl. It interferes with appressoria development and also effects conidia germination Takano *et al*., (2001) [34].

A similar result was found when conidia were treated with methanolic extract of *A. vasica* leaf (Fig. 5C) and stem (Fig. 5F).

Xu and Hamer (1996) [36] studied that PMK1 gene responsible for appressoria formation and infectious hyphae growth and found that the mutant PMK1 gene failed to appressoria formation but form swollen bodies of conidia. The result of our research was found similar to the above-mentioned research that conidia were germinated and form only germ tube might be mutated PMK1 gene due to phytochemicals of plant extracts.

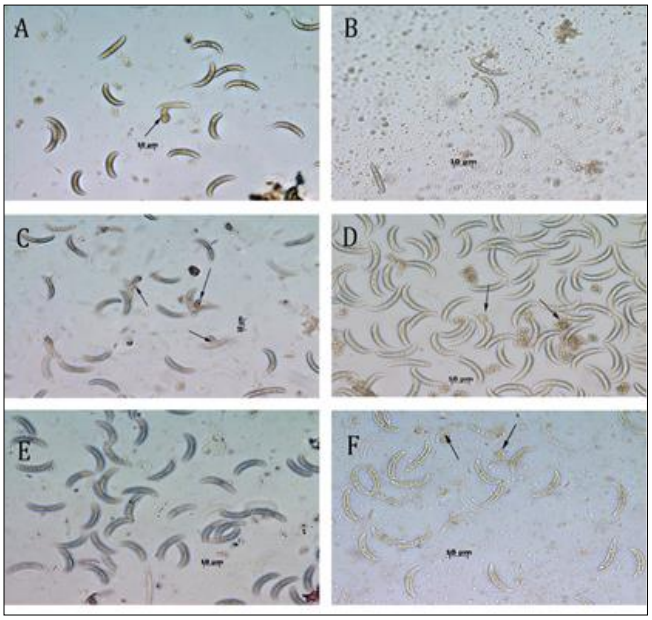

Fig 6: shows the effect of *A. indica* on conidial germination after 48 h (A) Ethanol leaf extract (0.5 g L^{-1}) on conidia germination. (B) Ethanol leaf extract (5 g L^{-1}) on conidia germination. (C) Methanol leaf extract on conidia germination. (D) Ethanol stem extract (0.5 g L^{-1}) on conidia germination. (E) Ethanol stem extract (4 g L^{-1}) on conidia germination. (F) Methanolic stem extract on conidia germination. Black arrow indicated appressoria formation and blue arrow indicate germ tube formation.

Flavonoid and Total Phenolic Content (TPC) in pathogen growth inhibition: Flavonoid content concentration of aqueous, ethanolic and methanolic extract of *A. indica* and *A. vasica* in figure 7 (A & B). The aqueous extract of *A. indica* and *A. vasica* possessedthehighest flavonoid concentration of 2.07 ± 0.003 and 2.86 ± 0.003 g/Kg among the leaf extract. Ethanolic and aqueous *A. indica* stem extract possessed an equal quantity of flavonoid concentration 1.97 ± 0.003 and 1.95 ± 0.002 g/Kg.

Total Phenolic Content (TPC) in *Acalyphain dica* and *Adhatoda vasica* were assessed and found different concnetrations of TPC which are depicted in figure 7 (A & B). In Aqueous, TPC in leaf portion of *A.indica* and *A. vasica* were found 157.22 ± 0.22 g GAE/Kg d.w. and 86.11 ± 0.55g GAE/Kg d.w. The crude methanolic stem extract of *A. vasica* has shown highest TPC with 105.66 ± 1.11 g GAE/Kg d.w., while the lowest TPC in the same species was crude extract dissolved in ethanolic extract with 55.00 ± 0.96 g GAE/Kg d.w. Similarly, the plant species *Acalypha indica* have comparatively higher TPC than *A. vasica* and the range of TPC within the species was 131.66 ±0.96 g GAE/Kg d.w.−192.52± 0.65g GAE/Kg d.w.

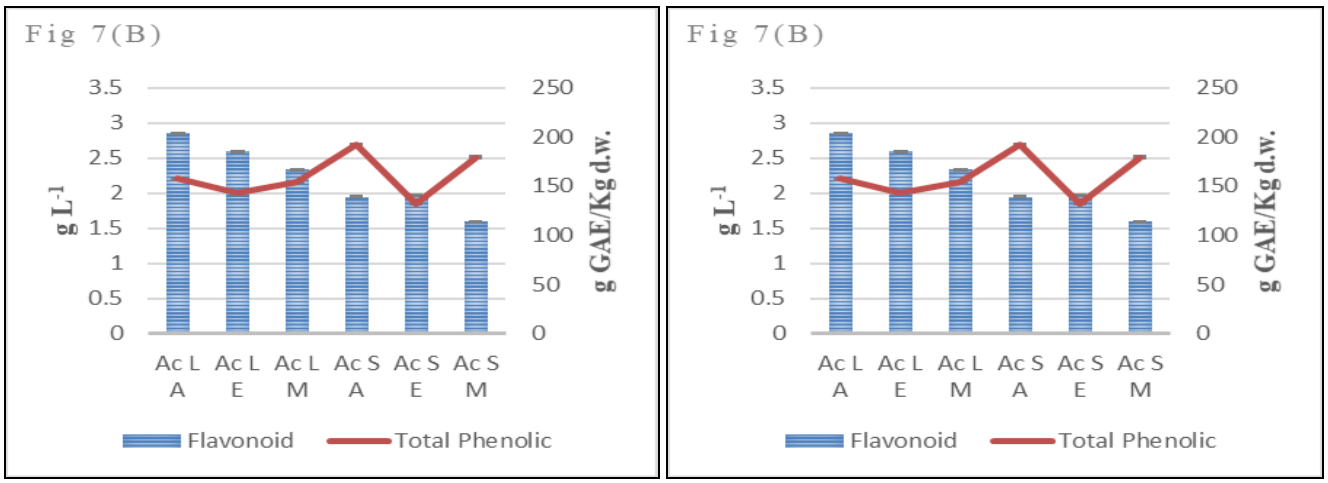

Fig 7: Depiet the Flavonoid content g/Kg and total phenolic concentration g GAE/Kg d.w**.** of *Adhatoda vasica* (A) and *Acalypha indica* (B). Ad = *Adhatoda vasica*, Ac. = *Acalypha indica* L=leaf, S=stem, A=Aqueous, E=ethanol, M=methanol.

Phenolic and flavonoid along with terpene play important role in antifungal activity, through disruption the permeability barrier of membrane structure in phenolic (Singburaudom, 2015) [29] impairing biosynthesis of ergosterin and disrupting membrane integrity, cell damage, induction of apoptotic DNA fragmentation, inappropriate ROS regulation (Da *et al*., 2019) hydrocarbons and oxygenated components of terpenespenetration to the fungi cell membraneandblocking synthesis of the cell wall, cytomembrane, cytoplasm and organelles (Kong *et al.* 2019) [15]. Mohamed *et al.*, (2017) [21] studied *Horwoodia dicksoniae*, *Citrullus colocynthis*, *Gypsophila capillaris*, *Pulicaria incisa* and *Rhanterium epapposum* plant extract against *Fusarium oxysporum*. *Pulicaria incise* was found strongly effective extract against fungus at 0.0092 g L^{-1} IC_{50} concentration while *C. colocynthis* was less effective.

Moreover, both plant extract had phenolic compound in higher concentration than flavonoid. Isolated phenolic compounds were similar but flavonoid (Quercetin and Rutin) was only in *P. incisa*. Additional, Quercetin were reported highly active against *F. oxysporum* than phenolic compound, ferulic acid, p-hydroxybezoic acid and caffeic acid. Beside this *P. incise* induces autophagosome formation in cell, plasmalemma distorted and structural disorganization in cytoplasma.Our result is also agreement with those of lee Sati *et al*., (2019) [27] who investigated thatkaempferol was found higher concentration in crud extract of *Gingo bioloba* than quercetin but quercetin and rutin was observed more effective than kaempferol when fungus was treated with standard quercetin, kaempferol and isorhamnetin. This result inform that activity of compound is depend on action mechanism, not concentration. Moreover, carvacrol and thymol are very closely similar in structure of phenolic, only in the difference of OH group position at benzene ring, show broad spectrum antifungal activity. Thymol expressed higher antifungal activity than carvacrol (Wang *et al.*, 2019) [28, 30]. Futhermore, modification of β-pinene with amide moieties and acylthiourea moieties, found changing of ethyl group on metha position of pinene improve the antifungal activity against *Colletotrichum gloeosporioides*, *Fusarium proliferatum*, *Alternaria kikuchiana* and *Phomopsis* sp. (Shi *et al.*, 2019) [28]. Sagbo *et al.*, (2017) [24] found similar findings that *Brachylaena elliptica* and *Brachylaenailici folia* contained 11.5 ± 5.05 and 8.86 ± 2.25 g/Kg flavonoid content, but both plants extract shown similar antimicrobial activity.

Conclusion and Future perspectives

Many researchers worked on the antibacterial, antifungal, antitumor, antidiabetic, antifertility and anti-inflammatory properties of *A. indica* and *A.vasica*. Some of these studies have been reported to identify the active principle of plants is responsible for such activities. However, no report is available on the antifungal of *A. indica* for increasing crop yield but very few for *A. vasica*. Some scientists have described both positive and negative results of the ethanolic extract of *A. vasica* on fungus growth inhibition. Leaf and stem extracts of both plants expressed the antifungal activity and MIC was obtained at 5 g L^{-1} concentration butthe highest antifungal activity was reported by the ethanolic extract than the methanolic extract. The highest conidia germination inhibition percentage and appressoria formation inhibition were observed by leaf ethanolic extract of *A. vasica*. According to the present study, flavonoid content was found higher in aqueous leaf extract than ethanolic and methanolic extract but the ehtanolic and methanolic extract delay the appressorium formation. Thus, ethanolic extract could be payed a role as the antifungal propertye due to the different flavonoid extracted or changing in the functional group. Further studies are needed to find the active compound of the *A. vasica* and *A. indica* that couldact as antifungal activity against the plant pathogens.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

Authors have expressed their gratitude to the School of Studies in Botany and Environmental Science, Jiwaji University, Gwalior (M.P.), for providing equipment during the tenure of research work. The authors also would like to thank the University Grant Commission, New Delhi (UGC), for a junior research fellowship for providing financial support as a fellowship.

References

1. Agostini JP, TimmerLW, Mitchell DJ. Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides f*rom citrus. Phytopathology,1992:82(11):1377-1382.
2. Akarsh S, Prashith TR, Ranjitha MC, Vidya P, Firdos GF. Inhibitory activity of some plants against *Colletotrichum capsici* and *Fusarium oxysporum* f. sp. *zingiberi*. J Medi Plant Stud,2016:4:165-168.
3. Alara OR, Abdurahman NH, Olalere OA. Ethanolic extraction of flavonoids, phenolics and antioxidants from Vernoniaamygdalina leaf using two-level factorial design. J King Saud Univ Sci,2020:32(1):7-16.
4. Bapat UC, Prabha S, Kumar J. Antifungal activity of ethanolic and petroleum ether extracts of some medicinal plants against the plant pathogenic fungus on Fresh Minimally Processed Vegetables. J Food Protec,2016:65:1909-1915.
5. Bhutia DD, Zhimo Y, Kole R, Saha J. Antifungal activity of plant extracts against Colletotrichummusae, the post-harvest anthracnose pathogen of banana cv. Martaman. Nut Food Sci,2016:46(1):2-15.
6. Buyu HA. Defining the Genetic Regulation of Appressorium Formation in *Cercosporazeae-maydis* (pulished thesis scholarworks@UARK). University of Arkansas, Fayetteville, 2018.
7. Da X, Nishiyama Y, Tie D, Hein KZ, Yamamoto O, Morita E. Antifungal activity and mechanism of action of Ou-gon (Scutellaria root extract) components against pathogenic fungi. Scientific reports, 2019:9(1):1-12.
8. De Corato U, Salimbeni R, De Pretis A, Avella N, Patruno G. Antifungal activity of crude extracts from brown and red seaweeds by a supercritical carbon dioxide technique against fruit postharvest fungal diseases. Postharvest Biol Technol,2017:131:16-30.
9. FAOSTAT. FAOSTAT database. Food and Agriculture Organization of the United Nations, Rome, Italy. FAO Statitical data, http://faostat.fao.orii/, 2018.
10. Fardin KM, Young MCM. Antifungal potential of *Avicennia schaueriana* S tap f & Leech. (Acanthaceae) against *Cladosporium* and *Colletotrichum*s pecies. Lett Appl Microbiol,2015:61(1):50-57.
11. Gogoi J, Barman I, Murrey E. Journey of capsicum from wild chili to world hottest chili (Naga chili). IJPBS,2018:8(3):835-840.
12. Herskowitz I. MAP kinase pathways in yeast: for mating and more. Cell,1995:80:187-197.
13. Ishnava KB, Chauhan KH, Bhatt CA. Screening of antifungal activity of various plant leaves extracts from Indian plants. Arch Phytopathol Pflanzenschutz,2012:45(2):152-160.
14. Jaihan P, Sangde K, Sangdee A. Disease suppressive activity of extracts from entomopathogenic fungus *Ophiocordyceps sobolifera* against chili anthracnose fungi *Colletotrichum* spp. in a pot experiment. J Gen Plant Pathol,2018: 84(3):237-242.
15. Kong Q, Zhang L, An P, Qi J, Yu X, Lu J *et al*. Antifungal mechanisms of α-terpineol and terpene-4-alcohol as the critical components of

Melaleuca alternifolia oil in the inhibition of rot disease caused by *Aspergillus ochraceus* in postharvest grapes. J appl microbial,2019:126(4):1161-1174.
16. Kumar A, Imran M, Kumar SPJ, Kumar M, Singh A, Srivastava Ak. Assessment of soil urease activity and its kinetic parameters in chilli rhizosphere under the influence of pesticides. Biopestic int, 2018: 14 (1): 45-57
17. Lahkar J, Goswami D, Deka S, Ahmed G. Novel approaches for application of biosurfactant produced by *Pseudomonas aeruginosa* for biocontrol of *Colletotrichum capsici* responsible for anthracnose disease in chilli. Eur J Plant Pathol,2018:150(1):57-71.
18. Liu X, Yan D, Ouyang C, Yang D, Wang Q, Li Y *et al*. Oils extracted From *Eupatorium adenophorum* leaves show potential to control *Phythium myriotylum*in commercially-grown ginger. PloS one,2017:12(5):p.e0176126.
19. Mesta RK, Kulkarni VR, Rao MSL. Identification of susceptible stage of fruit and role of biochemical constituents in fruit rot of chilli. Internat J agricSci,2007:3(1):46-48.
20. Mishra R, Rout E, Joshi RK. Identification of Resistant Sources against Anthracnose Disease Caused by *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* in *Capsicum annuum* L. ProcNatlAcadSci India Sect B BiolSci,2019:89(2):517-524.
21. Mohamed MS, Saleh AM, Abdel-Farid IB, El-Naggar SA. Growth, hydrolases and ultrastructure of *Fusarium oxysporum* as affected by phenolic rich extracts from several xerophytic plants. PesticBiochemPhys,2017:141:57-64.
22. Nduagu C, Ekefan EJ, Nwankiti AO. Effect of some crude plant extracts on growth of *Colletotrichum capsici* (Synd) Butler &Bisby, causal agent of pepper anthracnose. J Appl Bio Sci,2008:6(2):184-190
23. Radwan MM, Tabanca N, Wedge DE, Tarawneh AH, Cutler SJ. Antifungal Compounds from turmeric and nutmeg with activity against plant pathogens. Fitoterapia,2014:99:341-346.
24. Sagbo IJ, Afolayan AJ, Bradley G. Antioxidant, antibacterial and phytochemical properties of two medicinal plants against the wound infecting bacteria. Asian Pac J Trop Biomed,2017:7(9):817-825.
25. Sakthi SS, Geetha M, Saranraj P. Pharmacological screening of *Datura metel* and *Acalypha indica* for its antifungal activity against pathogenic fungi. IJPHR,2011:2(1):15-30.
26. Salam R, Devi PS, Sinha B, Bui R, Dinesh K, Chanu WT. In vitro Study on the Effect of Some Plant Extracts, Cow urine, Cow dung, Cow milk and Honey against *Colletotrichum capsici*. Int J Curr Micro biol App Sci,2018:7(6):2184-2191.
27. Sati P, Dhyani P, Bhatt ID, Pandey A. *Ginkgo biloba* flavonoid glycosides in antimicrobial perspective with reference to extraction method. JTCM,2019:9(1):15-23.
28. Shi Y, Si H, Wang P, Chen S, Shang S, Song Z *et al*. Derivatization of natural compound β-pinene enhances its in vitro antifungal activity against plant pathogens. Mol,2019:24(3144):1-15.
29. Singburaudom N. Hydroxychavicol from Piper betel leave is an antifungal activity against plant pathogenic fungi. J Biopest,2015:8(2):82.
30. Singh H, Zameer F, Khanum SA, Garampalli RH. Durantol-a phytosterol antifungal contributor from *Duranta repens* Linn. For organic Management of Sorghum Downy Mildew. Eur J Plant Pathol,2016:146(3):671-682.
31. Smith BJ, Black LL. Morphological, cultural and pathogenic variation among *Colletotrichum* species isolated from strawberry. Plant disease,1990:74(1):69-76.
32. Srikhong P, Lertmongkonthum K, Sowanpreecha R, Rerngsamran P. *Bacillus* sp. strain M10 as a potential biocontrol agent protecting chili pepper and tomato fruit from anthracnose disease caused by *Colletotrichum capsici*. Bio Control,2018:63(6):833-842.
33. Sutton BC. The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Common wealth Mycological Institut, 1980.
34. Takano Y, Oshiro E, Okuno T. Microtubule dynamics during infection-related morphogenesis of *Colletotrichum lagenarium*. Fungal Genet Biol,2001:34(2):107-121.
35. Wang K, Jiang S, Pu T, Fan L, Su F, Ye M. Antifungal activity of phenolic monoterpenes and structure-related compounds against plant pathogenic fungi. Nat prod Res,2019: 33(10): 1423-1430.
36. Xu JR, Hamer JE. MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. Genes development,1996:10(21):2696-2706.
37. Yeoh WK, Ali A. Ultrasound treatment on phenolic metabolism and antioxidant capacity of fresh-cut pineapple during cold storage. Food chem,2017:216:247-253.