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Research Article

EFFECT OF ORGANIC EXTRACTS OF SPICES ON PHYTOPATHOGENIC SPORULATING FUNGI

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ABSTRACT

The geographical area of Jharkhand has 29.61% of the total recorded forest area which possess diverse plants with significant secondary metabolites. Since use of synthetic pesticides are at extreme so the natural products like spices *viz*. *Allium sativum* (Garlic), *Myristica fragrans* (Nutmeg), *Piper nigrum* (Black pepper), *Terminalia chebula* (Chebulic myrobalan), *Trachyspermum ammi* (Ajwain), *Trigonella foenumgraecum* (Fenugreek) and *Zingiber officinale* (Ginger) of ethnomedicinal use were selected for antifungal efficacy. The ethyl acetate, ethanol and methanol extracts of selected plants were prepared at concentration 0.5,1,2,3,4 % and tested against 3 devastative sporulating fungi *i.e. Alternaria capsici*, *Curvularia lunata* and *Erysiphe pisi*. Against *E. pisi*; the ethyl acetate extract of all the seven spices showed 100% efficacy at 1% conc. while ethanol and methanol extracts of all spices was 100% effective at 3% concentration against *E. pisi*. In garlic each of the 3 organic extracts were 100% effective at concentration ranging from 2-4% against *A. capsici* while organic extracts of fenugreek failed to inhibit *A. capsici* spore germination. However, against *C. lunata* only the methanol extract at 3% conc. of myrobalan, ajwain and ginger were effective. *A. capsici* and *C. lunata* are deadly phytopathogens and their biocontrol through organic extracts of garlic, ajwain and myrobalan would be a better alternative to synthetic pesticides.

Keywords: Ethnomedicinal, *A. capsici*, *C. lunata*, *E. pisi*, Organic extracts, Biocontrol.

1. INTRODUCTION

Continuous use of synthetic pesticides had adverse effect on soil and humans health *via* plant produce. Even most pathogens develop resistance to several fungicides making the situation more futile by affecting the economy. Recent interests and advances in agriculture is towards the use of botanicals which not only be eco-friendly, healthy but also be commercially beneficial, natural and cultivated in large amount *viz*. Garlic (Liliaceae) has antimicrobial properties because of presence of high concentration of sulphur containing compounds [1-3]. Similarly, Thymol & Carvacol in ajwain (Apiaceae) aids in the digestion process by facilitating the release of gastric juice in intestine and possess antimicrobial properties [4]. Myrobalan (Combretaceae) popularly known as the king of medicines; its fruits, roots and bark had several traditional uses in folklore medicines [5]. Popular spices like Ginger rhizome (Zinziberaceae), and Nutmeg (Myristicaceae) its seed and mace are the favourites of Indian cuisine for enhancing flavor and taste. Different varieties of black pepper (Piperaceae) acting as anti-cancerous, antipyretic agent are commercially used to add taste in food, cure constipation, oral abscesses and toothaches. Fenugreek (Fabaceae), a common herb and spice is often used for its rich source of protein, fibre and minerals of dietary value.

C. lunata & *A. capsici* has been the most deadly phytopathogens since decades. Devastation caused by *Alternaria* spp. in commercial crops is approx 20% while its severity may reaches upto 80% of yield. *E. pisi* causes 20-30% crop loss which sometimes reaches to epidemic level. *C. lunata*; the leaf spot fungus and primarily invader of monocots makes yield loss upto 10-60% [6].

Present findings aims at the most potent spice among the selected botanicals viz. garlic, ginger, nutmeg, black pepper, chebulic myrobalan, methi and ajwain with antifungal properties which might be commercialized as natural alternatives to synthetic pesticides. For the screening of spices against sporulating fungi the ethyl acetate, ethanol and methanol extracts were prepared based on polarity.

2. MATERIAL & METHODS

2.1. Preparation of test fungi

The infected plant materials *i.e.* infected fruit of capsicum, leaf of paddy and leaf of pea plants were brought from experimental research farm of ICAR-RCER-RC, Palandu, Ranchi, Jharkhand to its laboratory and 5-10mm square lesions were cut containing both diseased & healthy tissue. The infected leaves were washed with sterilized distilled water. These were then kept in sterile moist chambers for 24 hrs for induced mycelial growth. Further identification & purification were done after transferring the individual fungal mycelia into PDA media. Following Riker and Riker (1936) [7] single spore isolation process; separation, maintenance and sub-culturing of *A. capsici, C. lunata* were done. One drop (20µl) of organic solvent plant extract or of known concentration was placed at the centre of clean & sterilized glass slide with the help of pipette (100µl). 1 drop of spore suspension was put on the same spot where the treatment was placed. After teasing or spreading the spore with sterilized needle, the treated fungus was incubated by placing the slides on petriplates containing moist blotters for 24hrs at 25±2°C for spore germination. After 24hrs using cotton blue stain the germination of spore or effect of plant solvent extract could be observed. Three replicates were used for each concentration of plant solvent extract. Total no. of spores germinated was noted at each concentration and every replication using Quebec colony counter. *i.e.* When the length of the germ tube exceeds half the diameter of the spores. The percentage of spores inhibited from germination was calculated by using the following formula [8]:

$$I = (C-T) \times 100/C$$

Where I= Inhibition percentage
C= No. of spores germinated in control
T= No. of spores germinated in treatment

2.2. Preparation of extracts

The healthy and non-infected plant parts such as bulb of garlic, rhizome of ginger, seeds of black pepper, nutmeg, fenugreek, myrobalan, ajwain were purchased from local market of Jharkhand. Garlic & ginger were peeled and along with other plant materials were rinsed in tap water to remove dirt while final washing were done in sterilized distilled water. The rinsed plant parts after shade drying for 24 hrs at room temperature (25±2°C) were macerated to fine powder in sterilized mortar & pestle [9]. For this cold extraction method was opted to extract the active ingredient present in these plant material [10]. The organic solvent & powdered plant parts were dipped in 1:1 ratio, kept for 7-9 days and routine shaking was done after every 24hrs. A biphasic layer was formed in which upper layer was fractionated. After fractionation solvent was evaporated in water bath at 60-62°C temperature to recover volatile materials & semi-solid materials were obtained from each extracts. The stock solution of the final extract was prepared.

$S_1V_1=S_2V_2$

Where, S_1= concentration of stock solution
V_1= Required volume to find out
S_2= Concentration to be prepared
V_2= volume to be prepared.

Five different concentrations of ethyl acetate, ethanol and methanol extracts were prepared using distilled water & pipette: 0.5%, 1%, 2%, 3%, 4% to evaluate the antifungal efficacy against the test fungi (seed borne).

3. RESULTS

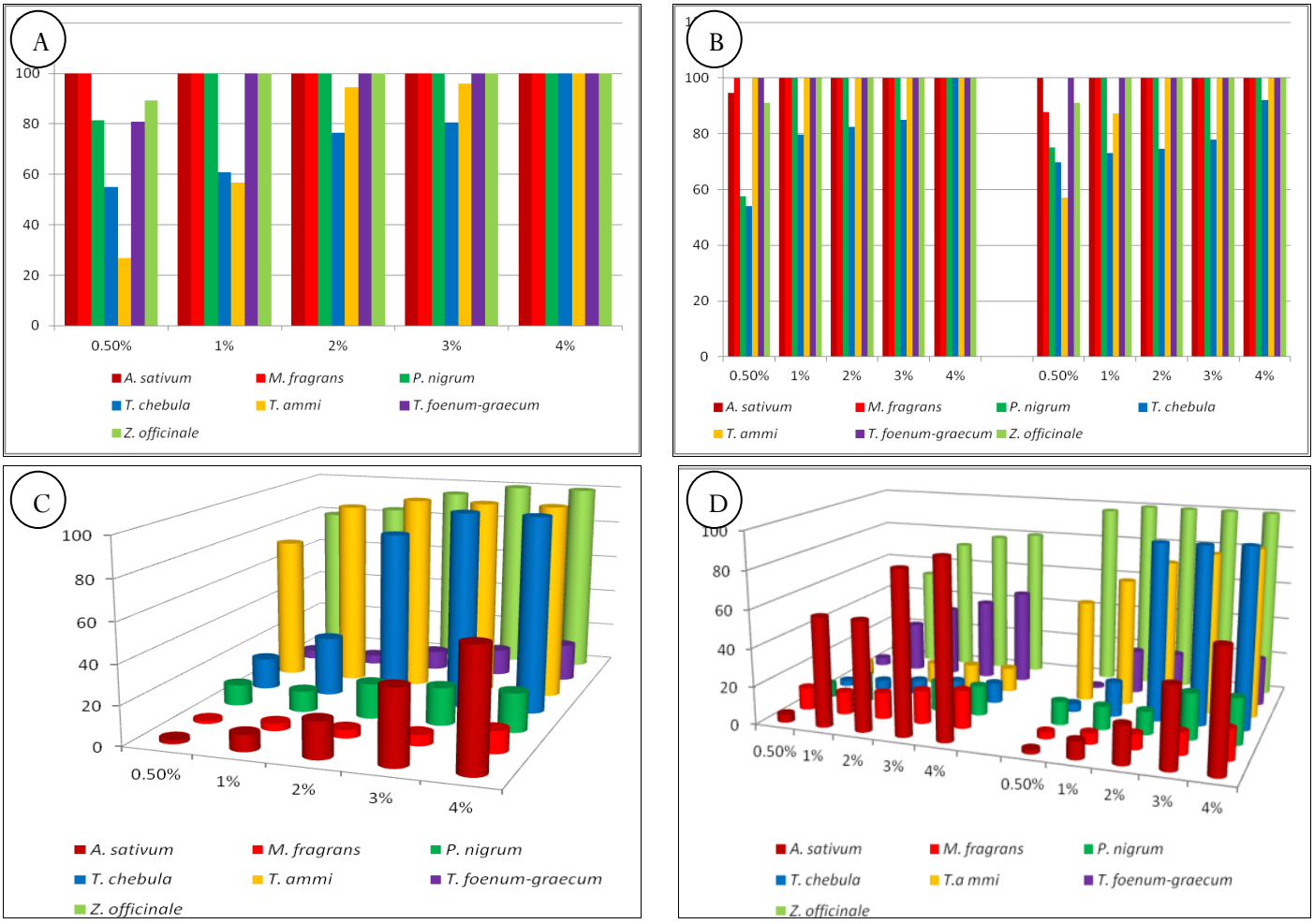
3.1. Antifungal efficacy of organic extracts of 7 selected botanicals/ spices against *E. pisi*, *A. capsici* & *C. lunata*

3.1.1. Efficacy of spices organic extracts against E. pisi

As compared to control which showed 30.66±2.96 spore germination in *E. pisi*, the ethyl acetate extract of garlic at 0.5% concentration showed 94.7% inhibition while from 1-4% there was 100% inhibition in spore germination of *E. pisi*. The ethanol and methanol extract of garlic also showed 100% inhibition of *E. pisi* at all test concentrations. Ginger at 0.5% ethyl acetate and ethanol extract showed 91.2% inhibition while at 0.5% of methanol extract 89.4% inhibition was observed. Similarly 1-4% concentrations of ginger ethyl acetate, ethanol and methanol extracts showed 100% inhibition against *E. pisi*. Similarly, the inhibition percentage of ethyl acetate, ethanol and methanol extract of black pepper at 0.5% was 57.5%, 75.1% & 81.5% respectively while from 1-4% black pepper showed 100% efficacy against *E. pisi*. Ajwain ethyl acetate extract showed 100% inhibition against *E. pisi* at all test concentrations but 100% inhibition was observed only at 2-4% of ethanol extract and 4% of methanol extract (Graph1:a, b).

Nutmeg ethyl acetate and methanol extract showed 100% inhibition against *E. pisi* at all test concentrations while in ethanol extract 100% inhibition was achieved at 1-4%. In myrobalan treatment 100% inhibition of *E. pisi* was achieved at 4% of ethyl acetate and methanol extract

while more than 50% inhibition was observed at 0.5-4% concentrations of ethanol extract. The ethyl acetate, ethanol and methanol extracts of fenugreek showed 100% inhibition of *E. pisi* at all test concentrations except for 0.5% of methanol extract where inhibition percentage was 80.9% (Graph 1:a, b).



Graph 1: Percentage inhibition of organic extracts of seven botanicals: a. Methanol extract against *E. pisi* b. ethyl acetate and ethanol extract against *E. pisi* c. methanol extract against *C. lunata* d. ethyl acetate and ethanol extract against *C. lunata*

Table 1a: Antifungal efficacy of ethyl acetate extract of spices against spore germination of *A. capsici*

Spices extracts	Efficacy of Ethyl acetate extract on spore germination					
	Control	0.5%	1%	2%	3%	4%
A. sativum	68.33±2.18 (0)*	0.0±0.0 (100)	0.0±0.0 (100)	0.0±0.0 (100)	0.0±0.0 (100)	0.0±0.0 (100)
M. fragrans	82.66±2.02 (0)	63.66±1.76 (22.9)	50.33±3.17 (39)	45.0±2.08 (45.5)	36.33±0.88 (56)	25.66±1.45 (68.9)
P. nigrum	58.33±4.84 (0)	40.66±1.76 (30.2)	36.33±0.88 (37.7)	15.0±1.73 (74.2)	7.33±0.33 (87.4)	6.33±0.33 (89.1)
T. chebula	82.66±2.02 (0)	76.66±1.76 (7.2)	72.66±1.76 (12)	64.66±2.60 (21.7)	61.33±1.85 (25.8)	57.33±1.20 (30.6)
T. ammi	82.66±2.02 (0)	15.0±1.0 (81.8)	6.0±0.57 (92.7)	0.0±0.0 (100)	0.0±0.0 (100)	0.0±0.0 (100)
T. foenum-graecum	82.66±2.02 (0)	81.66±2.02 (1.2)	75.33±1.45 (8.8)	72.66±1.76 (12)	67.66±1.45 (18.1)	65.0±0.57 (21.3)
Z. officinale	64.0±2.30 (0)	56.66±1.76 (11.4)	52.66±1.33 (17.7)	50.0±2.51 (21.8)	48.66±1.85 (23.9)	48.33±3.17 (23.9)

Table 1b: Antifungal efficacy of ethanol extract of spices against spore germination of *A. capsici*

Spices extracts	Efficacy of Ethanol extract on spore germination					
	Control	0.5%	1%	2%	3%	4%
A. sativum	87.0±2.5 (0)*	83.0±2.51 (4.5)	78.33±1.45 (9.9)	7.0±0.57 (91.9)	0.0±0.0 (100)	0.0±0.0 (100)
M. fragrans	82.66±2.02 (0)	68.33±0.88 (17.3)	56.66±1.33 (31.4)	33.33±3.33 (59.6)	15.33±4.84 (81.4)	10.0±2.64 (87.9)
P. nigrum	58.33±4.84 (0)	44.33±6.64 (24)	41.66±1.85 (28.5)	32.33±2.02 (44.5)	26.0±2.51 (55.4)	12.0±2.30 (79.4)
T. chebula	82.66±2.02 (0)	59.33±2.60 (28.2)	9.66±2.72 (88.3)	0.0±0.0 (100)	0.0±0.0 (100)	0.0±0.0 (100)
T. ammi	82.66±2.02 (0)	62.66±3.52 (24.1)	35.33±1.76 (57.2)	22.66±0.88 (72.5)	21.33±4.80 (74.1)	11.0±0.57 (86.6)
T. foenum-graecum	82.66±2.02 (0)	76.0±1.15 (8.05)	70.33±0.88 (14.9)	67.66±1.45 (18.1)	66.33±2.33 (19.7)	63.33±2.72 (23.4)
Z. officinale	66.0±1.52 (0)	55.33±1.76 (16.1)	48.33±3.75 (26.7)	47.33±0.88 (28.2)	41.0±2.51 (37.8)	39.66±3.17 (39.9)

Table 1c: Antifungal efficacy of methanol extract of spices against spore germination of *A. capsici*

Spices extracts	Efficacy of Methanol extract on spore germination					
	Control	0.5%	1%	2%	3%	4%
A. sativum	64.66±2.60 (0)*	14.66±1.76 (77.3)	12.33±4.48 (80.9)	0.0±0.0 (100)	0.0±0.0 (100)	0.0±0.0 (100)
M. fragrans	82.66±2.02 (0)	69.66±0.88 (15.7)	58.0±0.57 (29.8)	39.0±2.08 (52.81)	24.66±2.84 (70.1)	11.0±2.51 (86.6)
P. nigrum	58.33±4.84 (0)	38.66±1.3 (33.7)	37.33±1.20 (36)	25.66±0.88 (56)	22.0±2.08 (62.2)	18.66±3.75 (68.1)
T. chebula	82.66±2.02 (0)	56.0±2.64 (32.2)	12.0±1.15 (85.4)	9.66±1.20 (88.3)	0.0±0.0 (100)	0.0±0.0 (100)
T. ammi	82.66±2.02 (0)	62.66±4.8 (24.1)	24.33±2.33 (70.5)	19.66±1.20 (76.2)	19.0±2.08 (77)	10.66±1.76 (87.1)
T. foenum-graecum	82.66±2.02 (0)	80.66±1.20 (2.41)	77.0±4.93 (6.84)	70.0±5.03 (15.3)	66.66±0.88 (19.3)	63.33±2.02 (23.3)
Z. officinale	66.0±1.52 (0)	60.66±1.20 (9.09)	55.66±1.20 (15.6)	49.0±0.57 (25.7)	46.66±2.02 (29.3)	41.33±1.85 (37.37)

*()*in parentheses is showing % inhibition of spore germination*

3.1.2. Efficacy of spices organic extracts against A. capsici

Against control which showed 82.66±2.02 spore germination, garlic ethyl acetate extract showed 100% inhibition of *A. capsici* at all test concentrations while 100% inhibition was observed at 2-4% conc. of methanol extract and 3-4% of garlic ethanol extract (Table1:a, b & c).

Each of the ginger organic extracts failed to inhibit spore germination of *A. capsici* at all test concentrations (Fig1:g). Also, the ethyl acetate extract of ginger stimulated high mycelial branching of *A. capsici*. All the three organic extracts of black pepper showed more than 50% inhibition at 3-4% (Fig1:d),(Table1:a, b & c). .

At 2-4% Ajwain ethyl acetate showed 100% inhibition of *A. capsici* while maximum inhibition observed in Ajwain ethanol and methanol extracts at 4% were 86.6 and 87.1% respectively (Fig1:h).

In Ajwain, early initiation of branching in germ tube of spore in both ethanol and methanol extracts took place but true germination was lacking. More than 50% inhibition against *A. capsici* was observed in nutmeg ethyl acetate at concentration 3-4%, while in ethanol and methanol extracts inhibition was at 2-4% (Fig1:f). In 2% of nutmeg ethyl acetate extract branching of *A. capsici* appeared profusely (Table1:a).

Myrobalan ethyl acetate extract failed to inhibit spore germination in *A. capsici* but myrobalan methanol & ethanol extract at 3-4% was 100% effective (Fig1:e).

Each of the three organic extracts of fenugreek failed to inhibit the spore germination in *A. capsici* at all test concentrations and very high amount of branching in fenugreek ethyl acetate extract was observed (Fig1:c),(Table1:a, b & c).

Against fenugreek; *A. capsici* showed two types of germination: One in which the germ tube of *A. capsici* was thin which took blue stain when stained with cotton blue reagent and other which did not took stain.

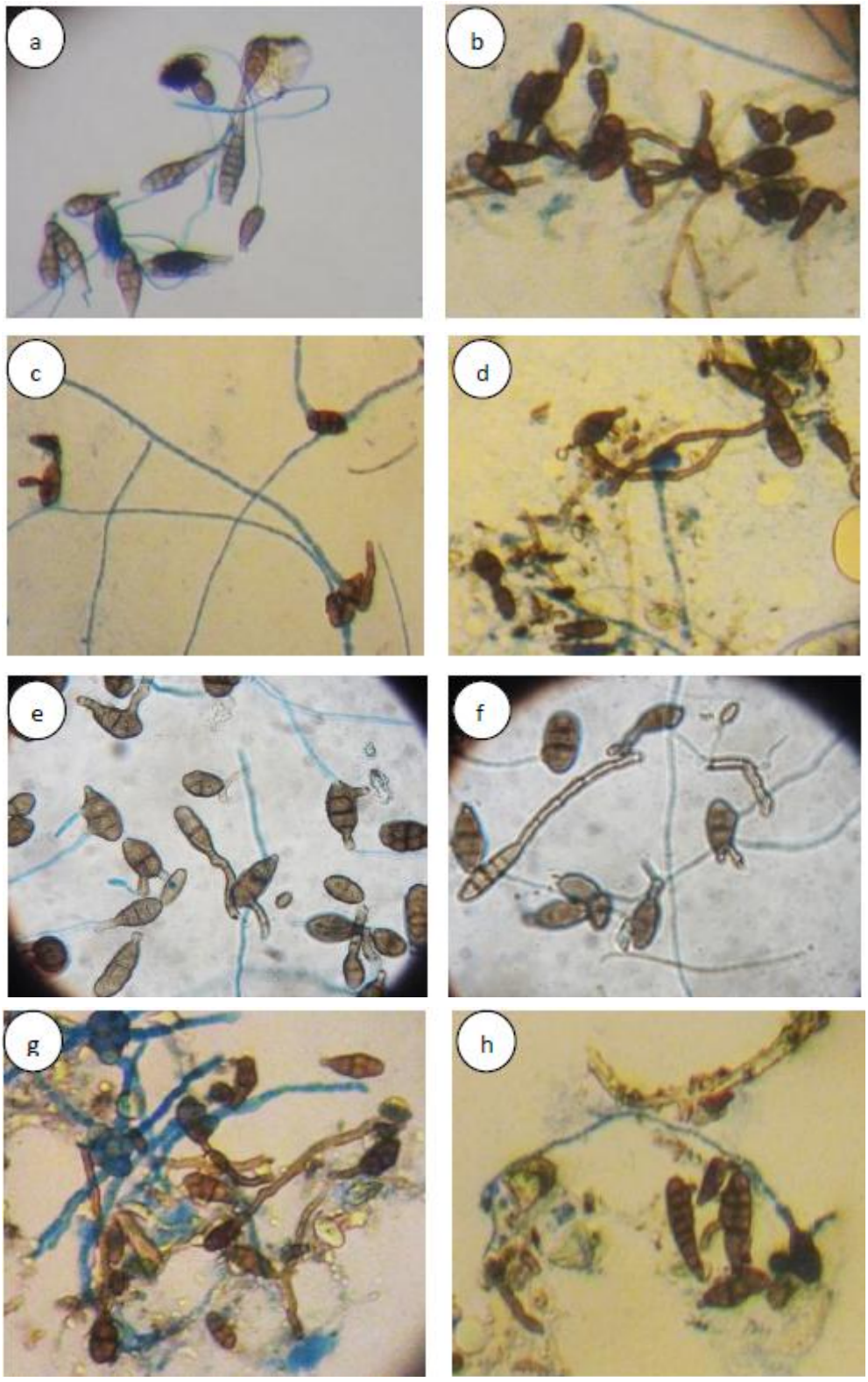


Fig. 1: Effect of methanol extract of spices against *A. capsici* at 4% concentration 1a. control 1b. Garlic 1c. Fenugreek 1d. Black pepper 1e. Chebulic myrobalan 1f. Nutmeg 1g. Ginger 1h. Ajwain

3.1.3. Efficacy of spices organic extracts against C. lunata

Garlic ethanol and methanol extracts at 0.5- 3% was ineffective against *C. lunata* (Graph1:c, d). However in garlic ethyl acetate extract more than 50% efficacy was observed at 1-4% concentration (Graph1:d). Ginger strongly inhibited spore germination of *C. lunata* in each of three organic extracts and almost all the test concentrations. In methanol extract of ginger, unipolar spore germination of *C. lunata* in 1% and bipolar germination at 0.5% conc. was observed. Each of the black pepper and nutmeg organic extracts showed least inhibition of *C. lunata* at all test conc. Against *C. lunata*, the ethanol extract of black pepper showed high spore germination and exceptionally much branching. Ajwain ethyl acetate was ineffective but its ethanol extract was 88.7% at 4% conc. and the methanol extract at 2-4%. showed 100% inhibition against *C. lunata* (Graph1:c, d).

Each of the myrobalan organic extracts was highly effective on *C. lunata* at 2-4% conc. (Graph1:c, d). At 0.5% conc. of ethanol extract of myrobalan; enormous germ tube growth with branching in *C. lunata* was observed which got reduced at 1%.

Similarly, all the three organic extract of Fenugreek failed to inhibit spore germination in *C. lunata*. The highest inhibition was 50.33% at 4% in Fenugreek ethyl acetate extract (Graph1:d). Fenugreek 1% ethanol extracts showed conidiophores (leaf like structure) in *C. lunata*.

4. DISCUSSIONS

Present findings go in accordance with previous research which illustrates that *M. fragrans* has antifungal properties [11, 12]. Present work confirms that the ethyl acetate, ethanol and methanol extracts contained the active components of *T.ammi* and *Z. officinale* which showed inhibition of the selected phytopathogens [13, 14]. Similar antimicrobial properties in the aqueous extract of both spices were also reported by several workers [15, 16]. The methanol extract of *T. ammi* was highly effective from 0.5-4% conc. which is in accordance with Behdani *et al.,* 2012 [17] who observed that by increasing Ajwain conc. from 250-500 µl, antifungal activity increases gradually.

However, *T. ammi* had been reported to inhibit more bacterial growth compared to fungi [18]. Active principle present in *P. nigrum* inhibited spore germination in *E. pisi* & *A. capsici* by more than 50% which supports the observations of Shukla and Dwivedi, 2012 [19] and relates to work by Bowers and Locke, 2000 [20] who studied that 5% aqueous pepper extracts reduced the population densities of *F. oxysporum* by 99.9%. Minimum efficacy of *T. foenumgraecum* against *A. capsici* & *C. lunata* might be due to its insolublization of active component in each of the organic extracts [9].

However, ethyl acetate extract of *T. foenumgraecum* at 4% conc. showed 50.33% inhibition of *C. lunata* showing antimicrobial properties which relates with the work demonstrated by Dharajiya et al., 2016 & Omezzine et al., 2014 [21,22]. Each of the organic extracts of *T. foenumgraecum* failed to inhibit *A. capsici* spore germination at all test conc. while Haouala et al, 2008 [23] investigated that *Alternaria* spp were sensitive to non-ground seed extract and ground seed extract of *T. foenumgraecum*. *A. sativum* was 100% effective against *E. pisi* & *A. capsici* at 3 - 4%. It may be due to metabolites present in garlic cloves which confers it the antifungal property [24-26]. Present research showed that *Z. officinale* was 100% effective at 3 & 4% against *C. lunata*; there are reports which suggests its 100% efficacy against other fungi [16] while Dwivedi and Sangeeta, 2015 [27] stated that on 4th day of inoculation *T. ammi* seeds inhibited *F. oxysporium* upto 81.43%. Moreover, present findings showed that except ethyl acetate extract on *C. lunata*; ethanol & methanol extracts of *T. ammi* showed more than 50% inhibition of all 3 test fungi at 1-4%.

Against *E. pisi* all the 3 organic extracts of all the seven selected spices were more or less 100% effective. Against *A. capsici*; *A. sativum*, *M. fragrans*, *P.nigrum*, *T. chebula* & *T. ammi* were highly effective. However against *C. lunata* only the ethylacetate extract of *A. sativum*, ethanol & methanol extract of *T. chebula*, *T. ammi* and *Z. officinale* were most effective. So these spices could be a potent alternative to synthetic fungicides.

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Conflict of interest

Authors declare that they do not have any conflicts of interest.

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