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# Evaluation of Antifungal Activity of Supercritical Fluid Extraction of *Ailanthus excelsa* against Powdery Mildew of Sunflower

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**ABSTRACT: Continuous usage of agrochemicals for the management of fungal diseases of plants has lead to the development of resistance towards the chemicals by fungi and ecosystem is under the treat. Hence biological methods are preferred with some advancements. *Ailanthus excelsa* which is usually called as tree of heaven is known to possess anti- fungal activity. The bio constituents such as flavonoids and phenols which are present in the leaves of *Ailanthus excelsa* is known to possess anti- bacterial, anti- fungal properties. The experiment was conducted to study the fungicidal activity of *Ailanthus excelsa* against powdery mildew of sunflower. For evaluating its activity against powdery mildew spore germination technique was followed for different concentrations of leaf extract of *Ailanthus excelsa*. Results revealed that maximum inhibition of spore germination of 98.37 per cent was observed in 10 per cent concentration which was on par with 9 per cent concentration of leaf extract of *Ailanthus excelsa* and the minimum inhibition of spore germination was observed at 1 per cent (28.78%) concentration in case of powdery mildew caused by *Golovinomyces cichoracearum*. Under field conditions, PDI of 7.41 per cent was observed after second spray of SFE of *Ailanthus excelsa* whereas, 9 per cent of SFE of *Ailanthus excelsa* showed 8.15 per cent of PDI which was on par with 10 per cent concentration of SFE of *Ailanthus excelsa*. At 1 per cent concentration of SFE of *Ailanthus excelsa* showed 50.71 per cent of PDI after second spray. The yield was higher at 10 per cent concentration of SFE of *Ailanthus excelsa i.e,* 8.68 q ha$^{-1}$.**

**Keywords:** Antifungal activity, Supercritical Fluid Extract, *Ailanthus excelsa*, Powdery mildew of sunflower.

## INTRODUCTION

Sunflower (*Helianthus annuus*) was domesticated as a food crop in North America, perhaps as early as 3000 BC. This was introduced to Europe in the 1600s and was successfully developed as an oil seed crop in Russia in the early 1800s. It is thought to have been domesticated 3000-5000 years ago by native Americans who would use them primarily as a source for edible seeds. They were then introduced to Europe in the early 16$^{th}$ century and made their way to Russia (Heiser, 1951). Sunflower is having very different characteristics over other oil seed crops owing to its wider adaptability to different agro-climatic conditions, highest oil production per unit area, short duration, excellent oil quality, high yielding potentiality, photoperiod insensitivity, lower seed rate and high seed multiplication ratio (Muhammad *et al*., 2012). This crop can also grown as inter crop with groundnut, pigeonpea, castor, soybean and urdbean. Since, it is a photo-insensitive crop, it can be grown throughtout the year.

In world, the cultivated area under sunflower is about 26 m ha and the production is about 56.96 million tonnes. In India, the cultivated area under sunflower is 4.006 lakh ha and the production is 2.840 lakh tonnes and the productivity is about 709 kg ha$^{-1}$ (Anon., 2020). Presently, Karnataka is the leading state in the country by contributing 64 and 52 per cent of total sunflower area and production respectively. It is the second important oilseed crop after groundnut having an area of 0.42 m ha with production of 0.28 m tonnes. However, productivity (547 kg ha$^{-1}$) is lesser than the national average of 709 kg ha$^{-1}$ (Anon., 2020).

Sunflower is a known host for over 30 pathogens, but the relative importance of specific diseases varies with geographic regions. Differences in climate, pathogen distribution and cropping practices affect the prevalence of individual diseases in each region.

In India, the powdery mildew was first reported in Bombay (Patel *et al.*, 1949) later in Rajasthan (Prasada *et al.*, 1968), West Bengal (Goswami and Dasgupta 1981) and Punjab (Bains *et al.*, 1998) causing a considerable reduction in yield. The pathogen signs on

leaves, originates as minute discoloured speck from which powdery mass radiates in all the sides of the leaves. Large area on the aerial parts of the host is covered with white powdery mass containing mycelia and conidia of the fungus (Singh, 1984; Amaresh *et al.*, 2013).

*Ailanthus excelsa* Roxb. (Simaroubaceae) is commonly known as Mahanimba due to its resemblance with neem tree. It is used as a folk medicine for variety of purposes like asthma, cough, cancer *etc*., (Kirtikar and Basu 1995). It consists of phytoconstituents such as quassinoids, alkaloids, protein flavonoids *etc.*, in different parts of the plants. It also has antifungal, antibacterial, antiviral, antimicrobial, antimalarial, antifertility, antitumour activities (Vaibhav *et al.*, 2014). The traditional claims, phytochemical investigations and pharmacological evaluations and some ayurvedic formulations provides the backbone to make this tree a tree of heaven. Quassinoids such as excelsin, glaucarubine, alianthinone glaucarubinone and glaucarubolone are found to show antifungal activity. The methanol extract of stem barks of *Alianthus excelsa* was partitioned with chloroform. The chloroform extract showed fungistatic and fungicidal activity against *Aspergillus niger*, *A. fumigatus*, *Penicillium frequentence*, *P. notatum* and *Botrytis cinerea*. So, present investigation was undertaken to know the efficacy of Supercritical Fluid Extract of *Ailanthus excelsa* as antifungal agent against powdery mildew of sunflower.

## MATERIALS AND METHODS

The efficacy of the Supercritical Fluid Extract of *Ailanthus excelsa* was evaluated against powdery mildew at different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 & 10%) along with tebuconozole (0.1%) by spore germination technique. Required concentrations of SFE extract of *Ailanthus excelsa* was prepared using distilled water. Two drops of super critical extract solution was taken on a clean cavity slide. *Golovinomyces cichoracearum* (DC.) V. P. Heluta spores were suspended in the SFE extract and cover it with cavity slide to avoid contamination and evaporation of water. Control treatment was maintained with distilled water. These cavity slides were kept in the Petri dishes lined with moist blotting paper and will be incubated at room temperature for 24 hours. After 24 hours, observations were taken at 10X microscopic fields for each cavity and the total number of spores and germinated spores in each microscopic field was recorded and per cent germination was calculated by using the formula given by Patil *et al*. (1997). The per cent inhibition of spore germination was calculated by formula given by Vincent (1927) for all concentration.

$$PG = \frac{A}{B} \times 100$$

Where,

PG = Per cent germination

A = Number of conidia germinated

B = Number of conidia observed

The average of three cavities (3 replications) was calculated and Per cent inhibition was calculated by formula

$$I = \frac{C - T}{C}$$

I= Per cent inhibition of spore germination

C= Germination of conidia in control

T= Germination of conidia in treatment

The field experiment was conducted at Main Agricultural Research Station (MARS) Raichur. The susceptible variety KBSH-44 was sown. The concentrations which were used in *in vitro* studies were also used in field conditions. The spray of these different concentrations of the chemical was carried out at two intervals *i.e.*, one spray before the initiation of the disease and the other spray at the interval of 15 days. The different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 & 10 %) of supercritical fluid extract of *Ailanthus excelsa* were prepared at the required quantities for the specified area and were sprayed at two intervals one spray before the incidence of disease symptoms and the other after 15 days.

Per cent disease index was calculated before spray, after first spray and second spray by following the disease scale given by Mayee and Datar (1986) for powdery mildew of sunflower. Later, Per cent Disease Index (PDI) was calculated using the formula given by Wheeler (1969).

$$PDI = \frac{\text{Sun of individual disease ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

## RESULTS AND DISCUSSION

Usually, synthetic fungicides are used in the management of plant diseases which are caused by fungi. But with the continuous use of the chemicals has led to the serious problem to human, environment and also led to the development of resistance of the pathogen against the fungicides which are applied at greater concentrations. Hence, the botanical which is extracted by supercritical fluid extraction process was evaluated against fungi.

The bioactive components which are obtained from *A. excelsa* includes flavones apigenin, apigenin 7-O- B glucoside, luteolin, luteolin 7-O-B- glucoside and flavonols kaemferol, kaemferol 3- O- a- arabinoside, keampferol-3 –O- arabinoside, kaempferol 3-O- B-galactoside, quercetin, quercetin 3-O- arabinoside. These compunds which are present in *A. excelsa* are reported to have anti-fungal activity by Rashed *et al.* (2013) and Poljuha *et al*. (2017).

In the present study, different concentrations of *A. excelsa* which was extracted by the process of supercritical fluid extract was used against the fungal pathogens *i.e., Golovinomyces cichoracearum* (DC.) V. P. Heluta which causes powdery mildew of sunflower.

The supercritical fluid extract of *A. excelsa* was obtained by following the standard procedure. From the concentrate which was obtained, different concentrations were prepared and were evaluated for its fungicidal efficacy against powdery mildew fungal pathogen of sunflower. The procedure was followed as mentioned in material and methods.

The results of studies on *in vitro* evaluation of different concentrations of supercritical extract against *G. cichoracearum* is presented in the Table. It revealed that, with increase in concentration, the per cent inhibition increases. The maximum inhibition of spore germination was 98.37 per cent was observed at 10 per cent concentration, next to this 94.28 per cent of inhibition of conidial germination was recorded at 9 per cent concentration, followed by 8 per cent concentration showed inhibition of about 92.33 percentage. Lowest inhibition was seen at one per cent concentration (28.78 %). Tebuconozole showed 73.46 per cent of inhibition which was on par with 5 per cent (78.59%) concentration of SFE of *A. excelsa*.

The identical results were obtained by Akhileshwari (2011), wherein they carried out *in vitro* evaluation of botanicals against powdery mildew of sunflower, wherein different botanicals like Nimbicidin, neem seed kernel extract, neem oil were used at three different concentrations *i.e*., 3, 5 and 7 per cent. Among all, 7 per cent concentration of nimbicidin showed 100 per cent inhibition of conidial germination.

The indistinguishable results were also obtained during the study which was conducted by Mahalakshmi and Alice (2013) where in *in vitro* evaluation of different plant extracts which included weeds, to study the efficacy in inhibiting the conidial germination of *E. polygoni*, 10 per cent concentration and different concentration of extracts like *Aillium sativum* showed the inhibition per cent of 93.90 per cent whereas 10 per cent concentration of *Prosopis juliflora* showed 91.23 per cent inhibition. Results obtained during the experiment, were closed to the results obtained by Dinesh *et al*. (2015), wherein the *in vitro* evaluation of different botanicals for its efficacy against powdery mildew of sunflower *i.e.*, Azadiractin and NSKE at 5 per cent concentrations showed 78.50 per cent and 72.38 per cent of inhibition of conidial germination. The experimental results obtained were in agreement with the observations made by Swetha (2016). They showed that, efficacy of seven botanicals at three different concentrations was carried out. Among all, garlic at 10 per cent showed the maximum inhibition of spore germination, which was followed by turmeric at 10 per cent concentration. Hence, with the increase in concentration, the inhibition also increased.

The results were in confirmation with the experiment carried out by Jadav and Kadvani (2019). About nine phytoextracts were tested against *Erysiphe cichoracearum* among which about six phytoextracts showed more than 50% inhibition. The highest spore germination inhibition (82.23%) was recorded at per cent concentration of neem followed by garlic at 10 per cent (80.69%).

From all the above studies, there is increase in inhibition of conidial germination with the increase in concentration of botanicals. The above study of supercritical fluid extract against powdery mildew showed the similar results as per these studies.

Since the use of chemical fungicides has increased in the present day agriculture, the ecology, human sustainability and food chain have greater changes. In order to reduce such effects, the use of botanicals which are natural has been carried out in this experiment and in order to utilize all the bioactive components present in the botanical *A. excelsa*, supercritical fluid extraction process was used, wherein particular temperature and pressure were maintained. At different concentrations these were evaluated under field, to know their efficacy. The procedure was followed which is mentioned in material and methods.

Under the field conditions, the results shows that, the minimum per cent disease index was 7.41 per cent at 10 per cent concentration which is on par with 9 per cent concentration (8.15%), 8 per cent concentration showed 10.38 per cent of disease index, further 7, 6, 5 per cent concentrations exhibited less than 25 per cent of disease index and the maximum per cent disease index was 64.31 under control. Tebuconozole, showed per cent disease index of about 23.21 per cent which was on par with 5 per cent concentration of SFE of *Ailanthus excelsa.* The results of the experiment is mentioned in Table 1.

The results obtained are in reflection with the investigation carried out by Hifsa Kiran and Shabeer Ahmad. (2005). In this experiment different phytobiocides like garlic, ginger, turmeric and neem oil were used under the field conditions in order to test their efficacy against powdery mildew of sunflower at 5 per cent concentration. Among all the phytobiocides, neem oil showed greater per cent disease control of about 47.34 per cent. The results were also alike to the experiment which was carried out by Surwase *et al*. (2009). In this experiment four different botanicals were evaluated against powdery mildew of sunflower. Among all, NSKE at 5 per cent showed greater efficacy. The research findings were in confirmation with the investigations which were carried out by Bademiyya and Ashtaputre (2020) against chilli powdery mildew. They evaluated seven botanicals at two different concentartions (2.5% & 5%). Among them, nimbicidin at 2.5 per cent showed (65.05 %) inhibition whereas at 5 per cent the inhibition percentage was (59.73%).

With the increase in concentration of SFE, the per cent disease index decreased. Hence, the yield was increased. The yield in case of control was about 5.06 q $ha^{-1}$ whereas in case of 10 per cent concentration of SFE of super critical fluid extract showed 8.68 q $ha^{-1}$ of yield.

Further there was no phytotoxicity of SFE extract was noticed under the field conditions with the increase in concentration upto 10 per cent. The plants did not show any scorching or rolling or burning effects due to SFE.

The obtained results were in confirmation with the investigations which were carried out by Meena *et al*., 2008; Venkataramanamma *et al*. (2020). The results pertaining to yield and increase of yield over control is mentioned in Table 2.

From the present investigation, with the increase in concentration of Supercritical fluid extract of *Ailanthus excelsa*, the inhibition percentage increased.

**Table 1: *In vitro* efficacy of SFE of *Ailanthus excelsa* against *Golovinomyces cichoracearum*.**

| Treatments | Inhibition of conidial germination (%) |
|---|---|
| T1 - 1%SFE | 28.78 (32.43)* |
| T2 - 2%SFE | 35.46 (36.54) |
| T3 - 3%SFE | 41.58 (40.15) |
| T4 - 4%SFE | 56.28 (48.60) |
| T5 - 5%SFE | 78.59 (62.44) |
| T6 - 6%SFE | 85.53 (67.67) |
| T7 - 7%SFE | 89.83 (71.44) |
| T8 - 8%SFE | 92.33 (74.06) |
| T9 - 9%SFE | 94.28 (76.22) |
| T10 - 10%SFE | 98.37 (82.70) |
| T11 – Tebuconozole | 73.46 (59.04) |
| T12 – Control | - |
| **C. D. at 1%** | **3.16** |
| **S. Em±** | **1.07** |

**Table 2: Field evaluation of different concentration of SFE of *Ailanthus excelsa* against Powdery mildew of sunflower.**

| Treatments | Before spray | First spray | Second spray |
|---|---|---|---|
| T1- 1%SFE | 13.12 (21.23) | 21.41 (27.56) | 20.71 (27.07) |
| T2- 2%SFE | 17.14 (24.45) | 13.74 (21.75) | 18.96 (25.81) |
| T3- 3%SFE | 22.90 (28.59) | 15.56 (23.49) | 14.14 (22.08) |
| T4- 4%SFE | 18.96 (25.81) | 11.85 (20.13) | 18.21 (25.26) |
| T5- 5%SFE | 19.00 (25.84) | 17.73 (24.90) | 19.22 (26.00) |
| T6- 6%SFE | 21.90 (27.90) | 14.81 (22.63) | 17.78 (24.93) |
| T7-7%SFE | 20.00 (26.56) | 12.23 (20.46) | 14.82 (22.64) |
| T8-8%SFE | 23.11 (28.73) | 11.15 (19.50) | 12.38 (20.60) |
| T9-9%SFE | 11.21 (19.56) | 10.15 (18.57) | 11.15 (19.50) |
| T10-10%SFE | 19.80 (26.42) | 10.15 (18.57) | 10.41 (18.82) |
| T11- Tebuconozole | 18.02 (25.11) | 28.89 (32.51) | 23.21 (28.80) |
| **CD** | **NS** | **3.25** | **3.38** |
| **SE(m)** | **NS** | **1.14** | **1.04** |

**Table.3. Evaluation of reduction of disease over control, yield and B: C ratio.**

| Treatments | Yield | Increase in yield over control (%) | B:C ratio |
|---|---|---|---|
| | | | |
| T1- 1%SFE | 5.73 | 13.24 | 1.28 |
| T2- 2%SFE | 5.97 | 17.98 | 1.32 |
| T3- 3%SFE | 6.00 | 18.57 | 1.36 |
| T4- 4%SFE | 6.23 | 21.12 | 1.39 |
| T5- 5%SFE | 6.63 | 23.02 | 1.41 |
| T6- 6%SFE | 6.93 | 26.25 | 1.47 |
| T7- 7%SFE | 7.35 | 26.95 | 1.51 |
| T8- 8%SFE | 7.71 | 32.37 | 1.58 |
| T9- 9%SFE | 8.25 | 33.04 | 1.63 |
| T10- 10%SFE | 8.68 | 52.34 | 1.75 |
| T11Tebuconozole | 7.18 | 26.89 | 1.56 |
| T12- Control | 5.06 | - | 1.12 |
| **CD** | **2.81** | | |
| **SE(m)** | **0.86** | | |

## CONCLUSION

With the increase in concentration of the supercritical fluid extract of *Ailanthus excelsa*, the inhibition of spore germination of *Golovinomyces cichoracearum* increased and also the per cent disease index of disease decreased. Also with the increase in concentration, there will not be any phytotoxic effect and yield increases with increase in concentration of supercritical fluid extract of *Ailanthus excelsa.*

## FUTURE SCOPE

As the fungicidal activity of *Ailanthus excelsa* is known, it can be further exploited with all other fungal diseases. Its fungicidal activity can also be exploited against the storage diseases. Also, its bactericidal and viricidal activity can also be exploited. Further, the efficacy of *A. excelsa* can be exploited for its insecticidal properties.

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