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Original Research Article

Antifungal Activity of *Moringa stenopetala* (Baker f.) Cufod Against *Alternaria helianthi*

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Abstract: Sunflower is an important oil seed crop in India. The crop is susceptible to various diseases among which leaf blight caused by *Alternaria helianthi* is more destructive. The present study aimed at determining antifungal potential of *Moringa stenopetala* (Baker f.) Cufod (Moringaceae) an indigenous plant to Ethiopia against *A. helianthi* isolated from sunflower leaves. Antifungal activity was determined by Poisoned food technique. The extract was effective and a dose dependent inhibition of mycelial growth of fungus was observed. At extract concentration 1.5mg/ml and higher, an inhibition of >70% was observed. The plant appears promising and the presence of bioactive principles in the extract might have accounted for the antifungal activity. The plant can be used in the management of leaf blight of sunflower.
Keywords: Leaf blight, Sunflower, *Alternaria helianthi*, *Moringa stenopetala*, Antifungal, Poisoned food technique.

INTRODUCTION

Sunflower (*Helianthus annuus* L.; Family Asteraceae) is one among the important oil seed crops in India and other countries. The crop suffers from various diseases such as leaf blight (*Alternaria helianthi*), rust (*Puccinia helianthi*), Powdery mildew (*Erysiphe cichoracearum*), Downy mildew (*Plasmopara halstedii*), Root rot (*Macrophomina phaseoli*), Collar rot (*Sclerotium rolfsii*) and Verticillium wilt (*Verticillium dahliae*). Among these, leaf blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is important one. The disease is recognized as potentially destructive in several countries such as India, Yugoslavia, Australia, Uganda and South Africa. In severe cases, the disease results in 70-80% yield loss [1-6]. The disease is destructive and causes reduction in flower size, number of seeds per head, seed weight and oil content. It can often lead to premature defoliation and stem lodge. The disease is characterized by typical dark brown to black, circular to irregular spots on leaves. On stem, petioles, sepals and petals, linear necrotic lesions are observed at different stages of growth. The disease affects the quality of seeds by affecting germination and initial vigour of the seedlings. The disease severity is known to increase or decrease depending on the environmental conditions during crop growth. It is more dangerous in tropical and subtropical regions due to favorable temperature and humidity [1,7,8,9].

Diseases caused by fungi represent the dominant group of plant diseases. These diseases are controlled by the use of synthetic fungicides. Interest in botanicals as antifungal agents is triggered due to drawbacks such as environmental pollution, high cost, development of resistance in fungi and toxicity to non-target organisms associated with inappropriate use of synthetic chemicals [4,10-13]. *Moringa stenopetala* (Baker f.) Cufod (Moringaceae) is an underutilized, fast growing, domesticated *Moringa* species indigenous to southern Ethiopia. It is an important indigenous vegetable food crop and contains high protein content. The seeds are promising as they possess flocculating property which can be exploited in purifying turbid water [14-17]. The plant is used traditionally as food and to treat malaria, hypertension, asthma, diabetes, common cold, wounds, retained placenta and stomach problem [18]. The plant possesses antileishmanial activity [18] and antitrypanosomal activity [19]. The plant is reported to exhibit antimicrobial [20-22], blood pressure lowering [23], anticoccidial [24], antihyperglycemic [25,26] and antihyperlipidemic activity [26]. The objective of the present study was to evaluate antifungal potential of leaf extract of *M. stenopetala* against *A. helianthi* isolated from leaf blight of sunflower.

MATERIALS AND METHODS

Collection and extraction of plant

The plant was collected form Ambo city located 112 km south of Addis Ababa, the capital city of Ethiopia. The plant was authenticated by referring regional flora **[27]**. The extraction was carried out by maceration process. The leaves were separated, washed well, dried under shade and powdered. 10g of leaf powder was transferred into a flask containing 100ml of methanol. The flask was sealed and left for 48 hours with occasional stirrings. After filtration through Whatman No. 1 filter paper, the filtrate was concentrated at 50^{o}C in an oven. The condensed extract was used for antifungal assay [22].

Isolation of fungus

The fungus *A. helianthi* was isolated from sunflower leaves showing typical symptoms of leaf blight. The surface sterilized leaves were cut into small pieces and placed on sterile Potato dextrose agar (PDA). The plates were incubated at 27±1^{o}C for until profuse growth of the fungus was observed. The fungus was then transferred into sterile PDA slant and purified through repeated subculturing. The identification of the isolate was made on the basis of cultural and microscopic characteristics. The pathogenicity test for the isolate was carried out by applying Koch's postulates. The seedlings of susceptible variety (KBSH-44) was inoculated with spore suspension of the isolate and the seedlings were incubated at ambient conditions namely temperature of 27±1^{o}C and relative humidity of 80% for the development of blight symptoms. Reisolation was made from the inoculated seedlings and the isolate completely resembled with the original culture in all respects [5].

Antifungal activity of leaf extract

Poisoned food technique was employed to screen antifungal activity of leaf extract of *M. stenopetala*. Different concentrations of leaf extract (0.1, 1.0, 1.5 and 2.0mg/ml of medium) were used to poison the sterilized and cooled (45^{o}C) PDA medium. The poisoned PDA media were poured into sterile petri dishes and allowed to solidify. 5mm mycelial discs were aseptically cut from 7 days old colony of *A. helianthi* on PDA plates and placed at the centre of control (without extract) and poisoned PDA plates. The plates were incubated at 27±1^{o}C for 5 days in upright position. The diameter of colonies developed on plates was measured in mutual perpendicular directions after incubation.

Antifungal activity of leaf extract (in terms of inhibition of mycelial growth of fungus) was determined using the formula:

Inhibition of mycelial growth (%) = (A – B / A) x 100,

where 'A' denotes diameter of colony on control plate and 'B' denotes diameter of colony on poisoned plate [22]. The experiment was conducted in triplicates. The results are presented as Mean±Standard Deviation (n=3).

RESULTS AND DISCUSSION

Members belonging to the genus *Alternaria* are cosmopolitan in nature. Species such as *A. alternata*, *A. solani*, *A. macrospora*, *A. porri*, *A. carthami* and *A. helianthi* are known to cause various diseases in their respective hosts [28-31]. *A. helianthi* is recognized as a destructive pathogen of sunflower. It has been shown that the use of chemicals, biocontrol microorganisms and plant extracts have shown to reduce the incidence leaf blight of sunflower and mycelial growth of *A. helianthi* [4,5,32,33]. In the present study, we determined the antifungal effect of leaf extract of *M. stenopetala* against *A. helianthi* by Poisoned food technique. This method is widely used to evaluate antifungal activity of various types of substances including plant extracts. If the sample has antifungal effect then reduction in the mycelial growth occurs in poisoned plate [5,31,34]. Table 1 and Figure 1 show the result of antifungal potential of *M. stenopetala* against mycelial growth of *A. helianthi*. Poisoning of the medium with extract resulted in reduction in mycelial growth of fungus. The inhibitory activity was concentration dependent. More than 70% inhibition of the fungus was observed at concentration 1.5mg/ml and higher. In a similar study, Waghe *et al* [5] showed dose dependent suppression of mycelial growth of *A. helianthi* by certain plant extracts among which marked activity was displayed by neem. In another study, Devi *et al*.[3] screened inhibitory effect of 20 plants against mycelial growth and spore germination of *A. helianthii*. Leaf extract of *Acalypha indica* displayed marked antifungal potential. More recently, we showed concentration dependent antifungal effect of methanol extract of leaf of *M. stenopetala* against three phytopathogenic fungi namely *Colletotrichum capsici*, *Fusarium oxysporum* and *Bipolaris sorokiniana* [22] and seed borne fungi [35].

Table 1: Colony diameter of *A. helianthi* in control and poisoned plates

Concentration	Colony diameter (cm)
0.0mg/ml	5.866±0.057
0.1mg/ml	5.333±0.057
1.0mg/ml	3.966±0.152
1.5mg/ml	1.733±0.115
2.0mg/ml	0.966±0.152

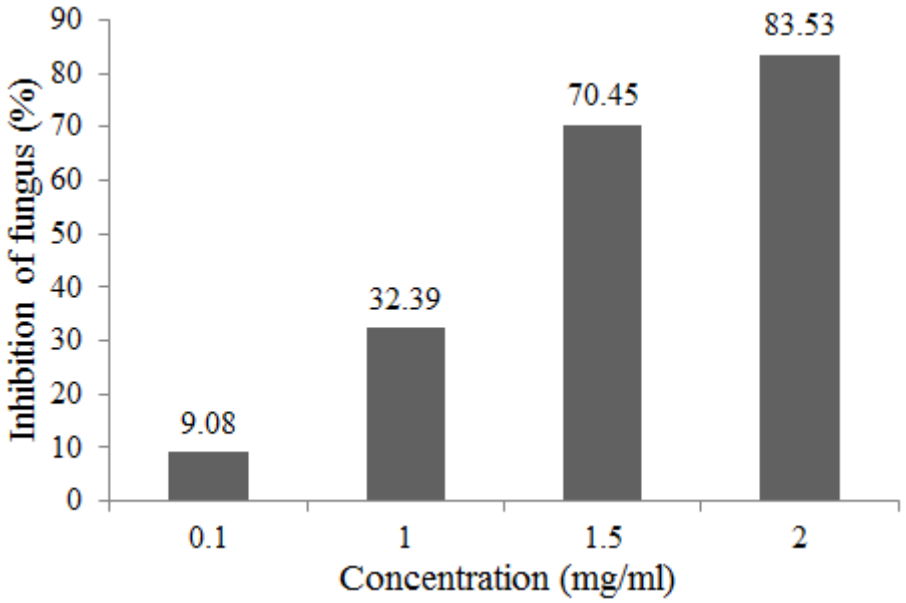

Fig-1: Extent of inhibition of *A. helianthi* by extract

CONCLUSION

Screening plants for antifungal activity against several phytopathogenic fungi as alternate strategy for plant disease control has been intensified nowadays due to problems associated with the use of synthetic fungicides. Botanicals appear to be cheaper and safer alternates for chemical agents as studies have shown the potential of botanicals to inhibit various phytopathogenic fungi. The result of the present study is promising as the leaf extract exhibited marked antifungal potential which may be attributed to the presence of bioactive principles in extract. Further studies are to be conducted to evaluate management of leaf blight in field conditions using *M. stenopetala* leaf.

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