Journal of Aquatic Biology and Fisheries Vol. 2/2014/ pp. 308 to 312

EFFICACY OF *SENNA ALATA* (L.) Roxb. LEAF EXTRACTS AS PLANT BASED FUNGICIDE AGAINST *ALTERNARIA SESAMI* INCITING SESAME LEAF SPOT

Lubaina, A.S*. and Murugan, K.
Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Trivandrum, 695 034, India.
**Email: lubainanizam@gmail.com*

Received on: 10 October 2013, accepted on: 12 December 2013

Abstract: Fungitoxic effect of *Senna alata* leaf extracts was evaluated *in vitro* on *Alternaria sesami*, the causal agent of sesame leaf spot. Sesame (*Sesamum orientale* L.) is grown as an important oil crop in all over world mostly in Indian subcontinent and Africa. Among the different diseases that attack sesame crop, Alternaria leaf spot has become a major disease causing significant reduction in yield. Keeping in view the drawback of chemical pesticides, the use of plant extracts in the management of plant diseases is gaining importance. Persual of earlier literature indicated that numerous attempts have been made in exploiting host resistance, modified cultural practices and fungicides. Induced resistance is an alternative to systemic disease resistance response of plants. Methodology includes hydro-distillation of *Senna alata* leaf using clevenger apparatus, plants were inoculated with conidial suspension of *Alternaria sesami* containing 1 x 10^3 conidia/ml for even distribution of the pathogen at 3 weeks after sowing (WAS) followed by spraying *Senna alata* extracts (10%) from 4WAS and repeated at one week interval until 6WAS under field experimental condition to evaluate disease severity and disease incidence, changes in the activity of defence related enzymes such as phenylalanine ammonia lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO) and total phenolic content after 24h from the last spray. A two fold activity of these defence related enzymes were observed in sesame after *Senna alata* leaf extract treatment which induce resistance against the pathogen infection in the host than the control. In field treatments aqueous extract of *S. alata* decreased the incidence and severity of disease in sesame. Hence there is scope to integrate *Senna alata* aqueous leaf extract for eco-friendly management of Alternaria leaf spot of sesame. Further work is required to increase the efficacy of the extracts in large ûeld and also to determine the biologically active ingredient present in extracts as well as its mode of action.

Key words: Alternaria leaf spot, Induced resistance, Inoculation, Phenylalanine ammonia lyase, Peroxidase, poly phenol oxidase, Phenol.

INTRODUCTION

Sesamum orientale L. is an ancient oil crop and it is probably the first oilseed crop used by human being, according to archaeological findings. Sesame has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible nutritive oil (48 to 60%) and protein (18 to 23.5%) (El-Bramawy and El-Sarag, 2012). Sesame oil is important in the food and pharmaceutical industry because of its distinct flavor (Elleuch *et al.,* 2007). The crop is highly drought tolerant, grows well in most kinds of soils and regions, and is well suited to different crop rotations. Sesame cultivars suffer considerable yield loss due to fungal diseases. *Alternaria* leaf spot of sesame has been identified as a predominant biotic pressure of single origin that limits seed yield both qualitatively and quantitatively.

Biocontrol involves artificial inoculation of microorganism to induce resistance and crop productivity. The research momentum generated by this practice in agriculture has revealed fascinating biology and led to copious fundamental discoveries. Future trends in biocontrol research will unite fundamental biology with the quest for solutions that will make biocontrol integral to the safe and wise management of agro ecosystems. Mostly biopesticides are used as curatives, so they may not perform as quickly as synthetic chemical pesticides. Similarly, biopesticides are generally less toxic to the user and also to non-target organisms, making them desirable and sustainable tools for disease management. *Senna alata* (L.) Roxb. known as candle bush or Empress candle plant, an evergreen ornamental

shrub having pinnately compound leaves with bright yellow cup-shaped flowers in axillary racemes and winged bean-like seed pods. It is native to South America and can be found widely in tropical region. The leaf extract exhibit various pharmacological properties such as anti-microbial, anti-fungal as well as anti-inflammatory activities (Okoro *et al*., 2010). Leaf sap contains a fungicide, chrysophanic acid used to treat several skin ailments. The aim of the present study was to evaluate the efficacy of *Senna alata* leaf extracts as plant based fungicide against *Alternaria sesami* causing leaf spot of sesame under field conditions.

MATERIALS AND METHODS

Plant materials

Sesame cultivar Thilarani (susceptible to *A. sesami)* seeds collected from Regional Agriculture Research Station, Kayamkulam, Kerala were used for the present study. Plantlets raised from seeds were maintained at 30°C in a temperature controlled glasshouse under a photoperiod of 12/12 h (light/dark), 60% RH and watered on every second day using a beaker without touching the foliage.

Collection and isolation of the pathogen

Leaves of sesame (*Sesamum orientale* L.) showing typical symptoms mainly on leaf blades as small, brown, round to irregular spots caused by *Alternaria sesami* were collected from University of Agricultural Sciences, Bangalore and the fungus isolated by the following technique indicated below. The infected leaves are cut into small pieces (2mm) were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 30 seconds and washed thrice in sterilized double distilled water to remove the traces of mercury and then transferred to sterilized petri plates (1-2 leaf bits per petri dish) containing potato dextrose agar (PDA).The Petri plates were incubated at room temperature (27±1°C) and observed periodically for the growth of the fungus. Fungal inoculum developed from the infected tissue was transferred to fresh PDA slants and incubated at 27±1°C for 12 days. The cultures were maintained throughout the study period by periodical transfers on sterile petri plates containing PDA medium under aseptic conditions to keep the culture fresh and viable. Sterile distilled water (10 mL) was added to the fungal cultures in each petri plate and the conidia were dislodged with a plastic rod to obtain a fungal suspension and were counted under microscope with the help of a hemocytometer containing 1×10^3 conidia mL^{-1}.

Ten *A. sesami* isolates were screened from different growing areas. The pathogen was identified up to species level based on their cultural and morphological characters. Further, the identification was confirmed from Indian type culture collection and identification, culture supply services, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

Preparation of Senna alata leaf extract

Healthy leaves of *Senna alata* collected were thoroughly washed in running water followed by rinsing with distilled water. It is then blotted with filter paper and was dried on mats under shade at room temperature, spread into thin layers over a drying period of 10-days. For pot and field experiments 10 g of powdered dried leaves *Senna alata* were hydro distilled in 100 ml sterile distilled water at 100°C for an hour and allowed to stand for 24 hrs at 4°C. The mixture was centrifuged at 4000 rpm for 10min. and the extract (10%) was filtered through Whatman No.1 filter paper into a sterile filter flask and stored in sterile condition for further use.

Field trials

Field trials were conducted on growing season of 2011 and 2012 respectively at Vembayam at the outskirt of the Trivandrum, Kerala, India. The field experiments in two years were laid using Randomized Complete Block Design (RCBD) in allocating treatments to plots and replicated three times. Each plots measuring 2.0 x 2.0m ($4m^2$) and separated by a clean space of 0.5 m^2 were used for assessment of disease severity and disease incidence. 20 g sesame seeds mixed with 100 g sandy soil was sown in 40 cm inter-row and 10 cm intra-row at 2 cm depth in each plot to achieve uniform distribution. The fields were inoculated with spore suspension of 1×10^3 conidia ml^{-1} for even distribution of the pathogen at 3WAS. *Senna alata* aqueous leaf extract (10%)

were sprayed as from 4WAS using pneumatic hand sprayer and repeated at one week intervals until 6WAS. *Indofil M-45 (Mancozeb) sprayed* at the recommended rate of *0.1% as a chemical fungicide.* Plants sprayed with sterilized water followed by conidial suspension of *A. sesami* served as control. The assessment of the number of infected plants was done using two permanent randomly placed quadrants (50 cm x 100 cm) per plot. The total number of plants and number of infected in a quadrant were counted and the percentage of disease incidence calculated. Observation on disease severity and disease reaction was recorded by assessing 10 randomly tagged plants per plot after stipulated treatment and recorded the overall score according to percentage area covered following 0-5 scale of Sheathe *et al.,* (2005) with some modifications. where 0 = no infection, 1= up to 5% area covered by the disease, 2 = 6-10% area covered, 3 = 11-20% area covered, 4 = 21-30% area covered, 5 = 31-100% area covered. This helped to determine the extent of establishment of the disease.

The following formula was used in determining the severity of infections.

$$\text{Disease severity} = \frac{\text{Sum of all scores} \times 100}{\text{Number of plants scored (N)} \times \text{Highest score (5)}}$$

Leaves from control and treated plants were sampled from pot and field treatment after 24, 48, 72, 96 and 120 h of last spray for estimation of phenylalanine ammonia lyase (PAL) (Anubhuti *et al.,* 2011), peroxidase (POX) (Popa *et al.,* 2009), poly phenol oxidase (PPO) (Mayer *et al.*, 1965) and phenolic content (Haddadchi and Gerivani, 2009). All the experiments were replicated thrice.

All data were subjected to analysis of variance, and there were significant differences ($p<0.01$), mean separation was done using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect on disease incidence and severity

By the application of *Senna alata* leaf extract under field conditions during the growing season of 2011 and 2012 reduced the disease incidence (number of lesions per leaf) and disease severity (percent of surface infected area) of *Alternaria* leaf spot disease in sesame (Table 1). Maximum severity of disease was observed in control (33%), whereas, in treated plants it was only 9%. A great reduction in disease incidence was observed in *Senna alata* leaf extract treatment (22.7) compared with control (high incidence - 58.2). Indofil M-45 substantially reduced the number of lesions and infected area on foliage and was statistically at par with that of *Senna alata* leaf extract treatment. This is consistent with the earlier reports that many plant products contain fungitoxic constituents that have the potential to control plant diseases (Owolade *et al.,* 2004; Enikuomehin and Peters, 2002). Results reported in the present study indicate that plant extract treatments not only suppressed the disease but also enhanced the growth and biomass of sesame plants compared to infected control (Data not shown).

Table 1. Effect of field spray with *Senna alata* leaf extracts on the incidence and severity of *Alternaria* leaf spot disease in cultivar Thilarani sesame during 2011 and 2012

Treatments	Disease Incidence	Disease incidence	Disease severity*	Disease severity*
	2011	2012	2011	2012
Senna alata	22.90^{b}	22.73^{b}	9.10^{b}	$9.13^{b,c}$
Mancozeb	18.20^{a}	18.77^{a}	6.20^{a}	6.73^{a}
Control	54.83^{c}	58.23^{d}	32.50^{c}	31.47^{d}
F	206.07	274.4	279.4	359
p-value	<0.01	<0.01	<0.01	<0.01

Values with different superscripts in the same column are significantly different ($p<0.01$) in Duncan's Multiple Range Test. *Percentage leaf area diseased estimated at 7WAS

Table 2. Effect of Senna alata treatment on peroxidase (POX), poly phenol oxidase (PPO), phenylalanine ammonia lyase (PAL) activity and phenolic content from 24 to 120 h of last treatment in treated (T) and control (C) Thilarani sesame leaves infected with A. sesami under field conditions

Hour	Treatment	24	48	72	96	120
POX	T	15.6a	17.2b	21.3d	19.9c	19.2c
U g^{-1} fresh weight	C	6.4a	8.6b	11.1d	9.4c	8.9b
PPO	T	8.1a	14.3d	12.5c	9.4b	8.2a
U mg^{-1} protein	C	3.5a	3.9b	4.4c	3.8b	3.9b
PAL nmole cinnamic acid g^{-1}	T	223a	352d	384d	292c	246b
fresh weight	C	108a	119b	128d	121c	113b
Phenol	T	1232a	1541c	1464e	1398d	1324b
µg gallic acid g^{-1} fresh wt	C	548a	571b	607d	589c	578b

Means within a row having the same letters are not significantly different according to Duncan's Multiple Range Test.

Application of chemical fungicides and emergence of fungicide resistant strains can be regulated by plant extract and bio control treatments in agriculture because of multiple reasons such as public concern regarding the health and environmental impacts of these toxic non biodegradable chemicals. The reduction in disease incidence and severity of sesame against *Alternaria* leaf spot with *Senna alata* treatment might be due to the antifungal activities towards the studied pathogen. These results agreed with the work of Hegazi and El-Kot (2010) in powdery mildew of *Zinnia*.

Peroxidase activity

Senna alata leaf extract treatment increased the level of POX in sesame leaves at all harvest times. A two fold increase in activity compared to control was observed in treated sesame after 24 h from the last spray. Maximum induction of POX activity was observed at 72 h of last treatment (21.3 U/g fresh weight). The enzyme activity was significantly increased up to 72 h and then it declined gradually whereas minimum peroxidase activity recorded in control treatments (Table 2). Peroxidase convert H_2O_2 to water provides an efficient system to prevent oxidative damage. Induction and accumulation of POX correlated with the onset of induced resistance suggest an active role for this enzyme in defense against pathogenic fungi and retard fungal growth (Jung *et al.,* 2004).

Phenylalanine ammonia lyase (PAL)

PAL is a key enzyme of phenyl propanoid metabolism leading to the synthesis of lignin, phenols, phytoalexins, and other compounds involved in a localized plant resistance process (El-Beltagi *et al.,* 2012). The activity of PAL was significantly higher (P>0.01) as compared to control in plant extract treated sesame. Highest activity of PAL was observed after 72 h of stipulated treatment 384 nmole cinnamic acid /g fresh weight whereas, activity at 96 h and 120 h was 292 nmole cinnamic acid /g fresh weight and 246 nmole cinnamic acid /g fresh weight respectively (Table 2). Kagale *et al.* (2004) reported higher activity of PAL in rice leaves treated with *Datura metel* leaf extract and inoculated with *Rhizoctonia solani* or *Xanthomonas oryza.*

Polyphenol oxidase (PPO)

Sesame plants inoculated with *A. sesami* followed by a plant based fungicide treatment induced an enhanced level of PPO. Increased PPO activity under biotic stress indicates its ability to induce resistance by producing defense compounds. During pathogenic stress PPO convert phenols into quinones. Highest level of PPO activity was noticed at 48 h of treatment (14.3 U mg^{-1} protein) and there after it decreased. Activity at 72 h of treatment was 12.5 U mg $protein^{-1}$, at 96 h and 120 h the activity decreased to 9.4 and 8.2 U mg $protein^{-1}$ respectively. Protection of tobacco and cucumber plants by leaf extracts of *R. sachalinesis* against powdery mildew have been reported to be accompanied by increased activities of polyphenol oxidase (Schneider and Ullrich, 1994).

Phenolic content

Increased level of phenolic compounds resulted in sesame leaves inoculated with *A. sesami*

followed by *Senna alata* leaf extract treatment. Maximum phenolic content (1541 µg Gallic acid g/fresh wt) was observed in sample harvested at 48 h and then it reduced gradually both in treated and control leaves (Table 2). Paul and Sharma (2002) reported a time dependent induction phenolics in barley upon treatment with aqueous neem leaf extract. Phenols have been suggested to play a role in plant resistance against many diseases. In addition to direct effects of phenols on fungal pathogen, phenolic compounds get oxidised to form more toxic quinones by peroxidase.

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