Exploring Biopesticidal Potentials of Leaf Extracts for Eco-friendly Management of Leaf Spot Disease of *Glycine max* (Soyabean)

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Abstract- **Plants have great potentials to inhibit fungal diseases, attempt is made to explore biopesticidal potentials of leaf extracts to combat *Alternaria* leaf spot disease of *Glycine max* / Soyabean, caused by *Alternaria alternata*. Four leaf extracts (*Cassia tora, Hyptis suaveolens, Ipomia carnia* and *Vitex negundo*) were used against test fungi, *Cassia tora* found very efficient at 25% concentration and remaining three leaf extracts inhibit fungi at 50% concentration. This is an Eco-friendly management of fungal disease.**

Key words: ***Biopesticide, Eco-friendly, Soyabean.***

I. INTRODUCTION

Conventional synthetic pesticides today pose threat of not merely potential but actual human injury and damage the environment to wit the almost ubiquitous presence of impermissible toxic residues in nearly all abiotic components of different ecosystem (Gupta and Gupta, (1979) pesticides residues are found in food, water and even milk. WHO estimated that each year there are 25 million cases of pesticide and as many as 20,000 unintentional deaths, primarily in developing countries (Devkumar and Dureja, 2002). Due to high pesticides residue level recently 130 containers of fresh grapes sent from India where rejected by Netherlands (Nag *et al*, 2004).

During last few decades, indiscriminate and unabated use of chemical insectides has resulted in several socio-economic problems, and as a result, the concept of integrated pest management (IPM) has come into existence. Different technologies are used under this program. Much of toxicity load of the planetary environment is due the long, widespread and continuous use of persistent and bio poisonous such as the conventional pesticides, herbicides, fungicides etc. giving rise to a distinct pesticide hazard syndrome, which is now too well to know need formal documentation.

Biopesticides offer one the best alternative for synthetic pesticides to tackle diseases of crop plants, biopesticides are certain type of pesticides derived from such natural material as plant parts. Fungicides or pesticides are very danger to ecosystem as their action remains mostly broad spectrum, which killing target and non-target organisms. But biopesticides are eco-friendly manages the crop diseases.

Leaf extracts of some very common plants have potentials to inhibit fungi or they have antifungal activities, so it is very easy method of manage fungal crop diseases and it is ecofriendly too. In the present investigation leaf extracts of very common plants (*Cassia tora, Hyptis suaveolens, Ipomia carnia* and *Vitex negundo*) manage Alternaria leaf spot disease of Soyabean (*Ghycine max*).

II. MATERIALS AND METHODS

Soyabean (*Glycine max*) a very common crop on which *Alternarai alternata* (Fr.) Keiss, a common fungus caused leaf spot disease, *Alternaria alternata* particularly, from the infected leaves of Soyabean (*Ghycine max*) was isolated from different districts of Maharashtra. The pathogen was maintained on Czapex Dox agar medium. Fresh leaves of (*Cassia tora, Hyptis suaveolens, Ipomia carnia* and *Vitex negundo*)were collected, washed, dried under shade and pulverized to obtain dry powder, for each plant extract 100gm powder, was taken. Extract of each plant was prepared with 95% ethanol (1:5 w/v) and condensed to serve as stock extract. The antifungal characters of stock extract were determined against the pathogenic test fungi Alternaria alternata. Following the poisoned food technique (Mishra and Tiwari, 1992) at 10, 25, 50 and 100% concentrations were made, petriplates containing Czapek Dox agar supplemented with different plant extracts at the four concentrations with three replications were inoculated with six mm disc of fungal mycelium, obtained from seven days old culture of fungal pathogen. The agar plates were kept upside down and incubated in BOD incubator at 28^{o}c $\pm$ 2^{o}c, the plates without leaf extracts were served as control. Radial growths of fungal colonies were measured at different intervals.

With the help of following equation, how much amount of conidia/ spore of test fungi were observed on treated portion of the host crop were calculated.

$$Spore/Conidia\ /Sclerotia = \frac{No.of\ spore\ /sclorotia/conidia\ observed\ per\ microscropic\ filed}{12.5} \times \frac{40{,}000}{1}$$

The percentage of capacity of leaf extracts, (Percentage Control Efficacy /PCE) to inhibited the growth of test fungi was calculated by following equation,

$$P.C.E. = 100(1 - \frac{X}{Y})$$

Where,
X, is the diameter of the lesion (mycelia growth) on biopesticide treated infected host
Y, the diameter of the lesion (mycelia growth) on untreated host (control)

In Vivo Studies:

In vivo studies was also carried out directly on the host crop plant (*Glycine max*/Soyabean).Leaf surface of the crop plant Glycine max were sterilized with alcohol and with the help of scalpel surface of leaves were damaged and the damaged surface of leaves were measured i.e. about 6 mm on each leaf and the damaged surfaces were treated with the biopesticides solutions (leaf extracts of test plants) with different concentrations. After 24 hours inoculation of pathogens was done on the damaged portions of leaves.

Inoculated damaged portions of leaves were covered with moist paper towel and were placed in the dark in laboratory temperature (26±3^{0}C) for 10 days, diameters of infected portions were considered for calculations as percentage control. Infected portions of leaves of host plants treated with sterilized water and mycelial suspension served as control.

TABLE I

Study of Effect of Leaf Extract (Biopesticides) on Growth of Test Fungi

Sr. No.	Biopesticides/ leaf extracts	Conc. %	Growth of *Alternaria alternata*
1	*Cassia tora*	10	23.66
		25	0.00
2	*Hyptis suaveolens*	10	35.33
		25	17.33
		50	0.00
3	*Ipomia carnia*	10	32.33
		25	14.66
		50	0.00
4	*Vitex negundo*	10	30.00
		25	16.33
		50	0.00
		Control	90.00

Table II

Study of Sporulation (No. of Conidia Per Microscopic Field and Per ml Suspension.) in Different Leaf Extracts.

Biopesticides		Conc. %	Sporulation of *Alternaria alternata*	
			No. of conidia per microscopic field	No. of conidia per ml.
1	*Cassia tora*	10	10.66	34112
		25	0.00	0.00
2	*Hyptis suaveolens*	10	18.00	57600
		25	9.66	30912
		50	0.00	0.00
3	*Ipomia carnia*	10	14.66	46912
		25	7.00	22400
		50	0.00	0.00
4	*Vitex negundo*	10	13.33	42656
		25	10.00	32000
		50	0.00	0.00
		Control	22.33	71456

TABLE III

In vivo Study (Antifungal Activity of Leaf Extracts on Host and PCE.)

Biopesticides		Conc. %	*Alternaria alternata*	
			Growth in mm on treated host	% control efficacy P.C.E.
1	*Cassia tora*	10	11.00	74.4
		25	0.00	100
		50	0.00	100
2	*Hyptis suaveolens*	10	13.00	69.7
		25	5.00	88.3
		50	0.00	100
3	*Ipomia carnia*	10	11.66	72.8
		25	4.00	90.6
		50	0.00	100
	Vitex negundo	10	12.33	71.3
		25	10.66	75.2
		50	0.00	0.00
		Control	43.00	0.00

III. RESULTS AND DISCUSSION

Biopesticides (leaf extracts) of all four plants with different concentrations (10%, 25%, 50% and 100%) were tested against the test fungi (*Alternaria alternata*) both *in vivo* and *in vitro*. The potentiality of leaf extract (biopesticide) of *Cassia tora* inhibited the test fungi totally at 25 % concentration and remaining three biopesticides i.e. *Ipomia carina*, *Hyptis suaveolens* and *Vitex negundo* inhibited test fungi at 50 % concentration, (Table No. 1) at 25% concentration the mycelial growth were observed, 14.66, 17.33 and 16.33 respectively.

Leaf extracts of four plants were also studies against the test fungi to know how much number of conidia was found in microscopic field with tested leaf extracts concentration wise and on the basis of that with the help of above formula number of conidia per ml suspension was get calculated. (Table No. 2). The leaf extract of Cassia tora showed lowest conidia per microscopic field (10.66) at 10% concentration only, the number of conidia get calculated in the same concentration i.e. 34112 (Table No.2). In the leaf extract of Vitex negundo, at 10% concentration number of conidia found13.33 and number of conidia per ml of suspension was calculated is that 42656, (Table No. 2). Remaining leaf extracts of two plants showed greater number of conidia both in microscopic field and per ml suspension.

In vivo percentage control efficacy (PCE) of every biopesticide against test fungi was also studied, leaf extract of *Cassia tora*, showed 100% percentage control efficacy at 25% concentration, followed to this, leaf extracts of *Ipomia carnia* showed 90.6% percentage control efficacy at the same % concentration i.e. 10% and remaining two leaf extracts obtained from *Hyptis suaveolens* and *Vitex negundo* showed 100% percentage control efficacy at 50% concentrations.(Table No.3)

According to Chitra *et.al*.,(2000) The leaf extract of *Datura inoxia* inhibited spore germination of *Clletotrichum capcici.* Dube and Dwiwedi (1991) also noticed that, the fungitoxic properties of plant extracts of *Acacia Arabica*, *Allium cepa* and *Allium sativum* on *Macrophomina phaseolina*. Pande, (1982), noticed that leaf extracts of Datura alba and *Canabis sativa* efficiently reduced the mycoflora associated with the seeds of *Eleusine coracana* in storage condition.

IV CONCLUSION

Alternaria leaf spot disease of *Glycine max* / Soyabean, caused by fungi *Alternaria alternata* is get managed with the help of four leaf extracts (*Cassia tora, Hyptis suaveolens, Ipomia carnia* and *Vitex negundo*) *Cassia tora* found very efficient at 25% concentration and remaining three leaf extracts inhibit fungi at 50% concentration. Rather than toxic chemical fungicides leaf extracts were used for controlling fungal disease. This is an Eco-friendly management of fungal disease.

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