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Efficacy of plant extracts against anthracnose (*Colletotrichum lindemuthianum*) of green gram (*Vigna radiata* L.)

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Abstract
Green gram is one of the important pulse crop and rich in protein content (18-31%) and play an important role in human and animal nutrition. *In vitro* studies were carried out using Garlic bulb extract, Neem leaf extract, Ginger rhizome extract, Dhatura leaf extract, Mehandi leaf extract against *Colletotrichum lindemuthianum*. Among the treatment Garlic bulb extract was found most effective and recorded significantly highest growth inhibition (80.56%) of *Colletotrichum lindemuthianum* as compared to treated carbendazim 0.1% (93.07%) and untreated control. Other plant extracts showed significant inhibition growth of *Colletotrichum lindemuthianum* were Neem leaf extract 10% (78.83%), Ginger rhizome extract 10% (74.38%), Dhatura leaf extract 10% (70.91 %) and Mehandi leaf extract 10% (64.60 %). In field evaluation a significant difference in the per cent disease intensity was observed. The minimum per cent disease intensity was recorded in Neem leaf extract 10% (23.57 %) as compared to treated carbendazim 0.1% (20.23%) and untreated control (37.60%), and other plant extracts showed the Garlic bulb extract 10% (26.86 %), Ginger rhizome extract 10% (28.85 %), Dhatura leaf extract 10% (32.31 %) and Mehandi leaf extract 10% (34.83 %). The most economical treatment which recorded highest cost: benefit ratio was the Neem leaf extract (C:B ratio, 1:1.69) as compared to treated carbendazim 0.1% (1:1.87) and untreated control (1:1.07). Other plant extracts such as Garlic bulb extract (1:1.40), Ginger rhizome extract (1:1.51), Dhatura leaf extrect (1:1.34) and Mehandi leaf extract (1:1.22)

Keywords: green gram, *Colletotrichum lindemuthianum*, plant extracts, carbendazim 50WP

Introduction
Anthracnose of green gram (*Vigna radiata* L.) caused by *Colletotrichum lindemuthianum*, is a serious disease in almost all green gram growing areas. In India, the green gram anthracnose was first reported from Jorhat of Assam state in 1951(Majid, 1953) [14]. Anthracnose diseases cause an estimated yield losses of 18.20 to 86.57 per cent. The per cent viability of conidia of *Colletotrichum lindemuthianum* in crop debris was significantly affected by duration of storage as well as different storage conditions. The conidia survival for a maximum of 360 days under freeze (4-5^{0}C) conditions and least survivability of 90 days under field condition (28-30^{0}C). (Kulakarni and Benagi, 2012) [11].

The disease is soil as well as seed borne. The fungus surviving as conidia and dormant mycelium. The conidia are hyaline, unicellular, falcate or lunette (sickle shaped) or cylindrical, more or less guttulate, muticate or with the apex prolonged into a simple cellular appendage, produced from phialides (conidiogenous cells) enteroblastically (Agrios, 2006) [1].

Botanicals are biodegradable and their use in crop protection is a practical sustainable alternative (Delvin and Zettel, 1999) [4]. It reduces environmental contamination and health hazards (Grange and Ahmed, 1988). Research on the active ingredients, fungicide preparation, application rates and environmental impact of botanical fungicides is a prerequisite for sustainable agriculture (Buss and Park, 2002). Botanical fungicides are unique because they can be produced easily by the farmers and small industries (Roy *et al.*, 2005) [20]. Few works have been done by using tobacco, neem, garlic. Antifungal activities of garlic, neem, allamanda have been reported by many researchers (Islam, 2005; Rahman *et al.*, 1999) Faruq *et al.*, (2015) [20, 19, 5].

Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the pathogen.

These groups of compounds show antimicrobial effect and serves as plant defence mechanisms against pathogenic microorganisms. The volatile antimicrobial substance allicin (diallyl thio sulphinate) is synthesized in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine Sulphoxide) mixes with the enzyme alliin-lyase. Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins Gurjar *et al*.,(2012) [6]. Plant extracts have been found useful and less harmful to man and animals as well as the environments reports, has shown that compounds from plant sources are moderately toxic and are suitable as fungicides. Neem (*Azdirachta indica*) showed significantly highest reduction in mycelial growth of the pathogen. The active chemical compound azardirachtin present in neem may be the reason for growth in inhibition of the pathogen Mishra *et al*., (2011) [13].

Indiscriminate use of chemicals is not only harmful to human beings but adversely affect the microbial population persent on the ecosystem. Hence use of plant extracts is recommended. Plants posses some substances in their parts like leave and bulbs which are toxic to many fungi causing plant disease. It is an effective, economical and ecofriendly methods of disease management. (Mahalakshmi *et al.*, 2013) [12]. Although chemicals are available for the management of *Colletotrichum* spp. a continuous inappropriate, non discriminatory use of chemicals is known to causes undesirable effects. Such effect are residual toxicity, resistance, environmental pollution and health hazards to humans and animals.(Ngullie *et al.*,2010) [16].

Materials and Methods

In vitro experiment

Isolation of pathogen

Green gram leaves with symptoms of anthracnose lesions were collected from field. The section of 4-5 mm was cut from the margin of the infected lesions and sterilized for one minute in 0.1% mercuric chloride solution and rinsed with several changes of Sterile Distilled Water. The sterile pieces were blotted on sterilized Petri plates containing solidified Potato Dextrose Agar (PDA) in aseptic conditions. The plates were incubated at ambient temperature (30±2°C) for 7 days after incubation according to the Nduagu *et al.*, 2008 [17].The tip of hyphal growth radiating from the infected tissue was transferred onto PDA. Culture was confirmed by microscopic examination and comparison with reference cultures (Boxter *et al.*, 1983) [3].

The plant extracts @10% *viz.* Garlic bulb extract, Neem leaf extract, Dhatura leaf extract, Mehandi leaf extract and Ginger rhizome extract were evaluated *in-vitro* against *Colletotrichum lindemuthianum* using Poisoned Food Technique and Potato Dextrose Agar according to (Nene and Tapliyal, 1993) [15]. Twenty ml of medium with desired concentration of plant extracts was poured in each sterilized petri plate. Suitable checks were kept for comparison. five mm mycelial disc of *C. lindemuthianum* was taken from periphery of ten days old culture and sclerotia taken from one month culture was placed at centre of the separate plates and All the treatment (inoculated) and control petri plates where then incubated at 24 ± 2°C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen. Observations on redial mycelial growth of *C. lindemuthianum* where recorded in each treatment and per cent growth inhibition of the test pathogen over control was worked out (Vincent, 1927) [24] as follows.

$$\text{Per cent inhibition (I)} = \frac{C - T}{\text{----------}} \times 100$$

Where, C

Percent reduction in growth of test pathogen

I = Percent reduction in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

In vivo experiment

Field preparation

An experiment was conducted during *Kharif* season of 2014 at the central research farm of Sam Higginbottom Institute of Agriculture Technology and Sciences. The selected field area was prepared by ploughed and harrowing during summer season, and the soil was pulverized and well decomposed farm yard manure was thoroughly mixed in the soil. Experimental plots were laid out as per statistical Randomized Block design. Total area was divided into 21 plots and plot size 2 x 1 m^2. To evaluate the efficacy of plant extracts against *Colletotrichum lindemuthianum* with Randomized Block Design in three replications. The crop was raised as per recommended package of practices and protective irrigation was given as and when required. A total plant extracts *viz.* Neem leaf extract 10%, Mehandi leaf extract 10%,Dhatura leaf extract 10%,Garlic bulb extract 10%,Ginger rhizome extract, and treated (carbendazim 50 WP (@ 0.1%), were evaluated under field condition.

Preparation of plant extracts

Healthy and disease free fresh leaf sample of selected plant species were brought to the laboratory and washed with sterile distilled water and then chopped into small bits with sterilized sharp knife. Each leaf sample was then separately grind and homogenized in mechanical grinder with equal quantity of sterile distilled water (1:1, w: v). The homogenate obtained was then strained through double layered muslin cloth and filtrate collected was then filtered through Whatman No. l filter paper using volumetric flasks (50 ml capacity). The clear leaf extracts obtained formed the stock solution of 100 per cent. An appropriate quantity of each leaf, bulb and rhizome extract was incorporated separately in the molten and cooled PDA medium in conical flask (250 ml capacity) to get desired concentrations 10 % of each extract according to the and autoclaved at 120 ºC for 15 minutes. (Onekutu *et al*., 2001) [18].

Three sprays of all the treatments were undertaken at intervals of 15 days, starting first spraying at 30 days after sowing of the crops. One plot/ replication was maintained as unsprayed control without receiving any plant extracts. Observations on foliage anthracnose disease were recorded after each sprayings and last observation on anthracnose were recorded at 15 days after last spraying. Disease severity of leaves was determined by the diagrammatic keys according to the scale described by (Singh, 2006) [23] per cent of the surface of the leaf affected by anthracnose. The infection on leaves were graded in 0-9 scale on the basis of severity of infection on leaves. The used scale was: 0 = no affected; 1 = 1% leaf area affected; 3 = 2-10% leaf area affected; 5 = 11-25% leaf area affected; 7 = 26-50 % leaf area affected; and 9 = 50 %>leaf areas affected. Five plants per treatment per replication were selected randomly and tagged for recording the observations. Three trifoliate leaves (bottom, middle and top) from main branch on each observation plant were selected for recording observations and per cent anthracnose disease intensity was worked out as detailed under table no 2.

Per cent disease intensity was calculated by using the following formula

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves/plant x Maximum disease grade}} \times 100$$

(Singh, 2006) [23]

Benefit Cost Ratio

Gross return was calculated by multiplying total yield with the market price of the produce. Cost of cultivation and cost of treatment imposition was deducted from the gross returns, to find out net returns and cost benefit ratio by following formula

$$\text{B: C} = \frac{\text{Gross return}}{\text{Cost of treatment}}$$

Were, B: C –Benefit and Cost ratio Hossain *et al.*, 2010) [8]

Results and Discussion

The data presented on inhibition Per cent of mycelial growth as influenced by plant extracts are given in table 1.The probable reason for such finding may be the volatile antimicrobial substance allicin (diallyl thio sulphinate) is synthesized in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine Sulphoxide) mixes with the enzyme alliin-lyase. Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins Gurjar *et al.*, (2012) [6]. Results indicated that all the plant extracts tested were found inhibitory and caused significant inhibition of mycelail growth of the test pathogen over treated (carbendazim) and untreated control. *In vitro* studies the Garlic bulb extract was found most effective and recorded significantly highest growth inhibition (80.56%) of the *Colletotrichum lindemuthianum* as compared to treated carbendazim 0.1% (93.07%) and untreated control, Other plant extracts showed significant inhibition growth of *Colletotrichum lindemuthianum* was recorded Neem leaf extract 10% (78.83%), Ginger rhizome extract 10% (74.38%), Dhatura leaf extract 10% (70.91 %) and Mehandi leaf extract 10% (64.60 %). Mehandi was found least effective and caused minimum inhibition (64.40 %) of the test pathogen. This result suggested by Jagtap *et al.* (2013), Mishra *et al*., (2011) [10, 13].

Table 1: Effect of plant extracts on radial growth and per cent inhibition of *Colletotrichum lindemuthianum* at 24 hrs, 48 hrs,72 hrs,96 hrs,120 hrs,144 hrs, and 168 hrs. After inoculation.

Treatment name	Mycelial radial growth (mm)							
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	Per cent inhibition
T_1 Dhatura leaf extract	13.7^{c}	17.9^{c}	20.3^{c}	22.9^{c}	26.1^{c}	30.1^{c}	33.8^{c}	70.91
T_2 Mehandi leaf extract	14.7^{b}	19.6^{b}	23.7^{b}	29.7^{b}	32.7^{b}	39.3^{b}	41.2^{b}	64.60
T_3 Neem leaf Extract	11.1^{d}	14.7^{d}	15.3^{d}	17.5d	18.9^{d}	20.3^{d}	22.1^{d}	78.83
T_4 Carbendazim	5.00^{f}	5.1^{f}	5.1^{f}	5.3^{f}	5.7^{f}	6.4^{f}	7.0^{f}	93.07
T_5 Ginger rhizome extract	12.5^{d}	16.2^{d}	18.7^{d}	20.8^{d}	23.7^{d}	25.8^{d}	27.1^{d}	74.38
T_6 Garlic bulb Extract	9.3^{e}	14.1^{e}	15.2^{e}	16.1^{e}	17.2^{e}	18.3^{e}	19.8^{e}	80.56
T_0 Control	39.2^{a}	76.4^{a}	89.8^{a}	90.0^{a}	90.0^{a}	90.0^{a}	90.0^{a}	00.00
F-Test	S	S	S	S	S	S	S	
SE.m+-	0.12	0.26	0.15	0.29	0.05	0.07	0.15	
CD at 0.05%	0.61	0.90	0.67	0.95	0.39	0.46	0.68	

The data presented on Per cent disease intensity influenced by plant extracts are given in table 2. The response of plant extracts against anthracnose of green gram as under field condition. The data indicated that all the treatments were significantly superior over control.

The probable reason for such finding may be neem (*Azdirachta indica*) showed significantly highest reduction in mycelial growth of the pathogen. The active chemical compound azardirachtin present in neem may be the reason for growth in inhibition of the pathogen Mishra *et al*.,(2011) [13]. A significant difference in the per cent disease intensity was observed the minimum per cent disease intensity was recorded in Neem leaf extract (23.57 %) as compared to treated carbendazim 0.1%(20.23%) and untreated control (37.60%),and other plant extracts show the Garlic bulb extract 10% (26.86 %), Ginger rhizome extract 10% (28.85 %), Dhatura leaf extract 10% (32.31 %) and Mehandi leaf extract 10% (34.83 %).and Mehandi leaf extract 10% (34.83 %) was least effective among all the treatments. This result suggested by Mishra *et al.*, 2011, Jagtap, *et al*., (2013) [13, 10]. The cost benefit ratio was worked out, interesting result was achieved. Among all the plant extracts used the best and most economical treatment was T_3 – Neem leaf extract (1:1.69), as compared to treated carbendazim 0.1% (1:1.87) and untreated control (1:1.07),and other plant extracts show the Garlic bulb extract (1:1.40), Ginger rhizome extract (1:1.51), Dhatura leaf extrect (1:1.34), Mehandi leaf extract (1:1.22). This result suggested by Gawade *et al*., 2009, Ngullie *et al*.,2010, Mishra *et al*.,2011 and Jagtap, *et al*., 2013) [7, 16, 13, 10].

Table 2: Effect of plant extracts on anthracnose (*Colletotrichum lindemuthianum*) disease intensity of green gram

Treatments	Per cent Disease intensity			B: C ratio
	30DAS	45DAS	60DAS	
T_1-Dhatura leaf extract	23.97^{bc}	26.65^{b}	32.31^{b}	1:1.34
T_2- Mehandi leaf extract	25.63^{ab}	27.58^{b}	34.83^{b}	1:1.22
T_3- Neem leaf extract	17.71^{ef}	21.29^{de}	23.57^{d}	1:1.69
T_4- Carbendazim (treated)	15.70^{f}	18.82^{e}	20.23^{e}	1:1.87
T_5- Ginger rhizome extract	21.62^{cd}	24.79^{bc}	28.85^{c}	1:1.29
T_6- Garlic bulb extract	20.10^{de}	22.87^{cd}	26.86^{c}	1:1.40
T_0- Control (untreated)	26.97^{a}	30.55^{a}	37.60^{a}	1:1.07
F-test	S	S	S	
S.Ed(+_)	1.17	1.47	1.24	
C.D(P=0.05)	2.56	3.20	2.69	

Conclusion

Based on the result it was observed that Foliar spray with Neem leaf extract 10 % proved to be most effective against anthracnose under field condition(compared to treated (carbendazim 0.1%) and untreated control) and Garlic bulb extract 10% was most effective in vitro condition followed by different plant extracts. In refers to the cost benefit ratio Neem leaf extract was more economical in minimizing the disease intensity by making less harm to environment.

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