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Evaluation Of Phyto Extracts Against Macrophomina Phaseolina Causing Root Rot Of Kalmegh (Andrographis Paniculata) In-Vitro And Pot Conditions

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Abstract
Kalmegh (Andrographis paniculata) is anannual, branched and herbaceous plant which is grown during kharif season in India.It is also known as "Bhui-neem" because of its similar bitterness as Neem tree. Whole plant parts of Kalmegh have medicinal properties, which are used to cure fevers, liver problems, diabetes, snake bites, jaundice,diarrhea, chronic malaria, and sore throat. Root rot of Kalmegh caused by Macrophomina phaseolina has affected significant yield loss. Major symptoms were seen yellowing and drooping of leaves on one side of the plant while the other part of the plant was found green. As the disease spreads, the entire plant turned yellow, wilted, and dried. Meanwhile, the collar region of the stem has splitted and become darkened. Maximum per cent growth inhibition was recorded in Allium sativum clove extract at 48 (57.41%), 72 (51.51%), 96(50.91%), 120 (52.74%), 144 (56.46%) and168 (57.50%) hours after inoculation. Under pot conditions, the minimum percent disease incidence was observed in Alliumsativum clove extract at 30 (4.44%), 60 (11.50%), 90 (16.57%) and 120 DAT (22.48%). All the treatments were significantly reduced disease incidence at 30 (84.49%), 60 (71.88%), 90 (68.94%) and 120DAT (65.38%). The mean per cent disease control was maximum in Allium sativum and clove extract @10% (72.67%).
Key words*: Phyto extract, Root rot, Macrophomina phaseolina, Kalmegh, in vitro.*

INTRODUCTION

Kalmegh (*Andrographis paniculata*) is popularly known as King of Bitters, it is a native plant of India and Sri Lanka that belongs to the Acanthaceae family with chromosome number 2n = 50. The plants' leaves and aerial parts have medicinal properties, which are used to cure fevers, liver problems, diabetes, snake bites, jaundice, diarrhea, chronic malaria, and sore throat. It is also used for leprosy, gonorrhea, scabies, boils and skin eruptions, chronic and seasonal fevers due to its "blood purifying" effect. Additionally, leaves and roots are utilized in advanced stages of gastric cancer, general debility, fever-related convalescence, and dyspepsia (Kumar*etal.,* 2012). Kalmegh is grown during rainy season (Kharif) in India. The plant requires hot and humid climate with plenty of sunlight. It is abundantly found throughout South Eastern Asia, including India, Sri Lanka, Pakistan, and Indonesia, However, plants are widely cultivated in China, Thailand, West Indies and Mauritius. It is extensively grown in plains of Uttar Pradesh, Madhya Pradesh, Chhattisgarh, West Bengal, Karnataka, Deccan, Assam, Gujarat, and Kerala in India. About 28 species of small annual shrubs in the genus *Andrographis* are found in tropical Asia. Among these species *Andrographis paniculata* is most popular for medicinal properties (Abhishek *et al.,* 2010 and Raina *et al*., 2013). Kalmegh is an annual, branched, herbaceous plant in a height of 30-110 cm, stem is abruptly quadrangular with many branches which are spreading widely. Flowers are white in colour with rose-purple dots on the petals. During the monsoon season, a well-maintained crop produces 3.5 to 4.0 tones dry herb per hectare (Niranjan *et al*., 2010).The National Medicinal Plants Board (NMPB) has reported in 2022, India had cultivated 27,000 hectares of land, resulting the production of 1,20,000 tons of herbage. Due to its numerous medicinal benefits in traditional Indian medicine the demand of Kalmegh is rising in India. However, area and production are stagnant due to unavailability of high yielding varieties and improved production and protection technologies.

Major insect pests of Kalmegh are Brown Scale (*Parasaisettia nigra)* and semilooper *(Panillaalbopenstata)* exhibited stunted growth and drying of plants and caused significant yield loss. Major diseases of Kalmegh are Root rot, Powdery mildew, Leaf web blight; Witches broom and Yellow vein leaf curl. Root rot caused by *Macrophomina phaseolina*is a destructive disease reducing yield losses up to 50% (Dave *et al.*, 2021). The initial symptoms are yellowing and drooping of leaves in one side of plant while other portion of the plant remains green. Subsequently entire plant becomes yellow, wilted, and dried as the disease spreads. Meanwhile, the collar portion of the stem is splitted and blackened. If severe infected plants were

uprooted, the lateral roots were found completely damaged and tap root revealed dryness, black with bark shredding (Thiribhuvanamala *et al.*, 2020). *Macrophomina phaseolina* is a fungus which belongs to Ascomycetes and exists in both sclerotial and pycnidial form. Its sclerotia are capable of withstanding high temperature and can remain viable in the soil and root debris for a period of 2 to 15 years. When the host tissues got decomposed, these sclerotia are released in clusters near the upper layers of the soil, where they can colonize both living and dead plant tissues. On the other hand, infected aboveground plant parts such as stems and leaves produce pycnidiospores, which are responsible for secondary dispersal of the fungus (Beas-Fernández *et al.*, 2006). The typical management strategies for control of root rot disease are crop rotation, enhancing soil fertility, utilizing resistant cultivars, employing bioagents and fungicides. The plant extract or botanicals have some antimicrobial, antifungal and antibacterial properties which make it an excellent treatment against plant pathogenic diseases. Botanicals extracts have some secondary volatile compounds such as azadirachtin in neem (Gopal *et al.*, 2007), quercetin in onion (Shafiq *et al.*, 2017), allicin in garlic (Tripathi and Lawande, 2006), gingerols and gingerdiol in ginger (Ficker *et al.*, 2003), and parthenin in Parthenium (Jai *et al.*, 2016). Keeping in view of the above Evaluation of phyto extracts against *Macrophomina phaseolina* causing root rot of Kalmegh *in-vitro* and pot conditions was undertaken in present investigation.

MATERIALS AND METHODS

Nine natural phytoextracts were examining their antimycotic effects on the mycelia growth inhibition of *Macrophomina phaseolina* using the poisoned food technique (Nene and Thapliyal, 1993). *Macrophomina phaseolina* infected Kalmegh Plants were carefully collected from the surrounding areas of Achary Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh-224229 (India) and washed thoroughly with tap water, and left to dry naturally. A hundred grams of the plant parts were ground using a pestle and mortar, while adding an equal amount (100 ml) of sterilized distilled water (in a ratio of 1:1, weight/volume). The resulting pulverized mixture was strained through cheesecloth, and the extracts obtained were subjected to centrifugation at 5000 rpm for duration of 20 minutes, forming a stock solution. The antifungal properties of the plant extracts *Viz;* T_1: *Allium cepa* bulb extract @ 10% concentration,T_2: *Allium sativum* clove extract @ 10% concentration,T_3: *Azadirachta indica* leaf extract @ 10% concentration,T_4: *Zingiber officinale* rhizome extract @ 10% concentration,T_5: *Parthenium hysterophorus* leaf extract @ 10% concentration,T_6: *Citrus aurantifolia* leaf extract @ 10% concentration,T_7: *Curcuma longa* rhizome extract @ 10% concentration,T_8: *Ocimum sanctum* leaf extract @ 10% concentration,T_9: *Lantana camara* leaf extract @ 10% concentration,T_{10}: Control were employed using poisoned food technique (Bambode and Shukla, 1973). Stock solution of each botanical extracts (10%) was mixed with 90 ml of Potato Dextrose Agar (PDA) media, separately, to achieve the desired concentrations. Then all the mixtures were sterilized in autoclave at 15 psi and 121 °c. After the sterilization, 20 ml of the aforesaid medium was carefully poured into sterile Petri plates and allowed to solidify. Actively growing culture of *Macrophomina phaseolina* was poured in each Petri plate at the centre with the help of cork borer (5mm diameter) under aseptic condition. One set of PDA Petri plate was maintained as control, only pathogen was poured without any plant extracts. The Petri plates were then placed in an incubator at room temperature (28±2°C) for 7 days and the radial growth was measured every 24 hours till the full growth of mycelium in control plate.

Percent growth inhibition:

Percent growth Inhibition = $\frac{C-T}{C} \times 100$

Where,

C = Mycelial growth diameter in control plate

T = Mycelial growth diameter in treatment plate

Growth rate of *Macrophomina phaseolina*

Growth rate (mm/day) = $\frac{Total\ final\ growth\ diameter\ (mm)}{Total\ number\ of\ days}$

In-vivo evaluation of phytoextracts (Pot experiment)

The experiment was conducted at Achary Narendra Dev University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh-224229 (India) during kharif-2022-23 using earthen pots with30 cm

diameter. Pots were filled with autoclaved 3.0kg soil pot^{-1} (soil: FYM, 3:1 autoclaved at 1.045 kg / cm^2 for 2 hours on three consecutive days). Before adding the pathogen in pots, it was multiplied on sorghum grains at 25±1°C temperature for one week and served as the soil inoculum. The top 5 cm layer of soil in each pot was thoroughly mixed with 20 g of inoculum, 7 days before transplanting of Kalmegh. The root rot susceptible genotype NDKL-10 was transplanted in the pots with three replications. The seedlings treatments were done *Viz;* T_1: *Allium cepa* bulb extract @ 10% concentration,T_2: *Allium sativum* clove extract @ 10% concentration,T_3: *Azadirachta indica* leaf extract @ 10% concentration,T_4: *Zingiber officinale* rhizome extract @ 10% concentration,T_5: *Parthenium hysterophorus* leaf extract @ 10% concentration,T_6: *Citrus aurantifolia* leaf extract @ 10% concentration,T_7: *Curcuma longa* rhizome extract @ 10% concentration,T_8: *Ocimum sanctum* leaf extract @ 10% concentration,T_9: *Lantana camara* leaf extract @ 10% concentration,T_{10}: Control by freshly prepared each aqueous plant extracts @10%concentration (volume/volume) for 15 minutes. After the treatment, excess water of seedlings was drained and air dried prior to transplanting.Total 15 seedlings was maintained in triplicate pots. After transplanting, pots were placed in green house. Foliar spray of the same phyto extracts was done at 60 DAT. The percent disease incidence and percent disease control were recorded at 30, 60, 90 and 120 DAT.

Per cent disease incidence (PDI)

The per cent disease incidence was calculated after first appearance of disease by adopting standard formula given by Bairwa *et al*., (2022).

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \times 100$$

Per cent disease control (PDC)

$$\text{Per cent disease control} = \frac{C-T}{C} \times 100$$

Where,

C = PDI in control

T = PDI in treatment

RESULTS AND DISCUSSION

Data presented in Table-1 revealed that all the treatments significantly inhibited the mycelial growth of *Macrophomina phaseolina* as compared to control. The maximum per cent mycelial growth inhibition was observed in *Allium sativum* (57.41%) followed by *Allium cepa* (48.23%), *Parthenium hysterophorus* leaf extract (36.53%), *Azadirachta indica* leaf extract(29.45%), *Ocimum sanctum* leaf extract (24.27%), *Lantana camara* leaf extract (19.69%), *Curcuma longa* rhizome extract (13.42%), *Zingiber officinale* rhizome extract (11.55%) and *Citrus aurantifolia* leaf extract(8.81%) at 48 hours after inoculation. In case of 72 hours after inoculation, the maximum mycelial growth inhibition was found in *Allium sativum* (51.51%) followed by *Allium cepa* (42.70%), *Parthenium hysterophorus* leaf extract (35.56%), *Azadirachta indica* leaf extract (32.26%), *Ocimum sanctum* leaf extract (26.12%), *Lantana camara* leaf extract (19.36%), *Curcuma longa* rhizome extract (14.06%), *Zingiber officinale* rhizome extract (12.27%) and *Citrus aurantifolia* leaf extract(9.07%). After 96 hours of inoculation, highest mycelial growth inhibition was found in *Allium sativum* clove extract (50.91%) followed by *Allium cepa* bulb extract (46.38%),*Parthenium hysterophorus* leaf extract (45.61%), *Azadirachta indica* leaf extract (40.08%), *Ocimum sanctum* leaf extract(37.76%), *Lantana camara* leaf extract (29.97%),*Curcuma longa* rhizome extract (25.09%), *Zingiber officinale* rhizome extract (23.75%) and *Citrus aurantifolia* leaf extract(20.01%). In case of 120 hours after inoculation, the maximum mycelial growth inhibition was found in *Allium sativum* (52.74%) followed by *Allium cepa* (48.82%),*Parthenium hysterophorus* leaf extract (45.93%),*Azadirachta indica* leaf extract(44.39%), *Ocimum sanctum* leaf extract(40.16%), *Lantana camara* leaf extract (31.32%),*Curcuma longa* rhizome extract (31.13%), *Zingiber officinale* rhizome extract (30.54%) and *Citrus aurantifolia* leaf extract(24.30%). After144 hoursofinoculation, maximum per cent mycelial growth inhibition was found in *Allium sativum* clove extract(56.46%) followed by *Allium cepa* bulb extract (49.69%),*Parthenium hysterophorus* leaf extract (48.38%),*Azadirachta indica* leaf extract(47.28%), *Ocimum sanctum* leaf extract(43.40%), *Lantana camara* leaf extract (36.30%),*Curcuma longa* rhizome extract (35.56%), *Zingiber officinale* rhizome extract (33.12%) and *Citrus aurantifolia* leaf extract(25.93%). In case of 168 hours after inoculation, the maximum mycelial growth inhibition was found in *Allium sativum* (57.50%) followed by *Allium cepa* (51.04%),*Parthenium hysterophorus* leaf extract (49.69%),*Azadirachta indica* leaf extract(47.28%), *Ocimum sanctum* leaf

extract(43.82%), *Lantana camara* leaf extract (39.55%),*Curcuma longa* rhizome extract (34.50%), *Zingiber officinale* rhizome extract (32.28%) and C*itrus aurantifolia* leaf extract(26.05%). The finding of experiments was supported by Savaliya*et al*., (2015), in which they reported that garlic clove (*Allium sativum*) extract has been found significantly superior in terms ofinhibition of mycelial growth of *Macrophomina phaseolina* (Tassi.) Goid, which causes sesame root rot followed by *Allium cepa* bulb extract, because garlic extract contains a unique class of organo sulfur compound called allicin. Allicin inhibits germination of spores and growth of hyphae due to having antifungal and antibiotic properties (Marchese *et al*., 2016). Similarly, *in vitro* condition nine phytoextract were tested against *Macrophomina phaseolina* by poison food technique which resulted maximum inhibition of mycelial growth and sclerotia formation by garlic (*Allium sativum*) extract followed by *Allium cepa* (Palat *et al*. 2004). Garlic have some bioactive derivatives that destroy integrity of cell membrane and lead to death of the fungal cell (Low *et al*., 2008).Onion contains important antimicrobial phytochemical flavonoid compound viz., Quercetin, which causes disruption of the plasma membrane, inhibits nucleic acid synthesis, protein synthesis and mitochondrial functions of the fungus. Similarly, weed plants such as Parthenium contains 35 lactones of the pseudoguaicinolide and xanthanolide skeletal groups. It has contained Parthenin as a major constituent which exhibits antifungal activity (Mughal *et al*., 1996 and Ramesh *et al*., 2003).A common medicinal plant *Azadirachta indica* contains Nimbin which may act as a potent biocide because it shows fungitoxic effect against several fungal phytopathogens viz, *Aspergillus* and *Rhizopus* (Mondali*et al*., 2009).

Table-1: Efficacy of phyto extracts on percent mycelia growth inhibition of *Macrophomina phaseolina in-vitro* conditions

Treatments		Concentration (%)	Per cent mycelial growth inhibition over control						Mean
			48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.	
T_1	*Allium cepa*	10	48.23 (43.65)	42.70 (40.80)	46.38 (42.91)	48.82 (44.32)	49.69 (44.82)	51.04 (45.59)	47.81
T_2	*Allium sativum*	10	57.41 (48.98)	51.51 (45.86)	50.91 (45.52)	52.74 (46.57)	56.46 (48.71)	57.50 (49.31)	54.42
T_3	*Azadirachta indica*	10	29.45 (32.37)	32.26 (34.60)	40.08 (39.27)	44.39 (41.78)	47.28 (43.43)	48.46 (44.11)	40.32
T_4	*Zingiber officinale*	10	11.55 (18.90)	12.27 (20.87)	23.75 (29.15)	30.54 (33.54)	33.12 (35.13)	32.28 (34.62)	25.66
T_5	*Parthenium hysterophorus*	10	36.53 (36.78)	35.56 (36.60)	45.61 (42.48)	45.93 (42.66)	48.38 (44.07)	49.69 (44.82)	43.62
T_6	*Citrus aurantifolia*	10	8.81 (16.18)	9.07 (17.49)	20.01 (26.54)	24.30 (29.52)	25.93 (30.61)	26.05 (30.68)	19.03
T_7	*Curcuma longa*	10	13.42 (20.64)	14.06 (21.99)	25.09 (30.04)	31.32 (34.02)	35.56 (36.60)	34.50 (35.97)	26.47
T_8	*Ocimum sanctum*	10	24.27 (28.95)	26.12 (30.73)	37.76 (37.90)	40.16 (39.32)	43.40 (41.20)	43.82 (41.44)	35.92
T_9	*Lantana camara*	10	19.69 (25.68)	19.36 (26.08)	29.97 (33.18)	31.13 (33.90)	36.30 (37.04)	39.55 (38.97)	26.78
T_{10}	Control	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
SE(m)±			1.07	0.96	1.03	0.60	0.46	0.95	-
CD (P=0.01)			3.21	2.89	3.09	1.80	1.38	2.86	-

*Figures in parentheses are arc sin transformed values

Result of experiment (Table-2) revealed that the all treatments were significantly reduced the disease infection. The per cent disease control was maximum in *Allium sativum* (84.49%), followed by *Allium cepa* (77.74%), *Parthenium hysterophorus* (68.78%), *Ocimum sanctum* (61.52%), *Azadirachta indica* (54.54%),

Curcuma longa (39.06%),*Lantana camara* (30.30%), *Zingiber officinale* (23.69%)and *Citrus aurantifolia* (15.95%) at 30 DAT. In case of 60 DAT maximum per cent disease control was observed in *Allium sativum*(71.88%), followedby *Allium cepa* (66.63%), *Parthenium hysterophorus* (58.60%), *Ocimum sanctum* (50.29%), *Azadirachta indica* (45.84%), *Curcuma longa* (34.02%),*Lantana camara* (29.87%),*Zingiber officinale* (23.65%)and *Citrus aurantifolia* (18.35%). Similarly, at 90 DAT maximum Per cent disease control was observed in *Allium sativum* (68.94%), followed by *Allium cepa* (62.78%),*Parthenium hysterophorus* (53.24%), *Ocimum sanctum* (50.82%), *Azadirachta indica* (41.16%), *Curcuma longa* (36.86%),*Lantana camara* (29.72%),*Zingiber officinale* (24.89%)and *Citrus aurantifolia* (21.05%). At 120 DAT highest Per cent disease control was recorded in *Allium sativum*(65.38%), followedby *Allium cepa* (58.84%),*Parthenium hysterophorus*(51.00%),*Ocimum sanctum* (46.97%), *Azadirachta indica* (40.93%), *Curcuma longa* (37.37%),*Lantana camara* (29.68%),*Zingiber officinale* (25.78%)and *Citrus aurantifolia* (23.35%).Findings are also similar with theresults of Lakhran and Ahir, (2020)in which they tested the efficacy of plant extracts against dry root rot of chickpea under *in-vivo* conditions and observed that maximum per cent disease control over the check wa in 64.24% at 40 DAS and 68.00% at 60 DAS in Garlic clove extract.Kumar and Chaudhary, (2020) also evaluated seven plant extracts on charcoal rot of Soybean caused by *Macrophomina phaseolina*. The maximum per cent disease control 65.2% was observed in garlic (*Allium sativum*) clove extract over the check. Basically, garlic has contained allicin compound which showing inhibitory effects against fungi which are inhibiting thiol containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups (Cavallito and Bailey, 1944).Quercetin and phenols arefound in onion bulb extracts andare effective against *Botrytis cinerea*, *Rhizoctonia solani*, *Alternaria alternata*, *Fusarium culmorum*, *Phytophthora cactorum* and *Ascosphaeraapis* (Olszowy *et al*., 2022).Tan *et al*., (2021) evaluated nine limonoids against nine phytopathogenic fungi i.e.,*Fusarium oxysporum*, *Magnaporthe oryzae*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Alternaria* spp., *Botrytis cinerea*, and three *Phytophthora* species to check their antifungal effectiveness. The broad-spectrum

Fig.1: Lab Experiment

Fig.2: Kalmegh trial in pot conditions

Table -2: *Effect of phyto extracts on per cent disease control under pot conditions*

Treatments		Concentration (%)	Per cent Disease Control (PDC)				Mean
			30 DAT	60 DAT	90 DAT	120 DAT	
T_1	*Allium cepa*	10	77.74 (61.93)	66.63 (54.76)	62.78 (52.44)	58.84 (50.11)	66.49
T_2	*Allium sativum*	10	84.49 (66.93)	71.88 (57.95)	68.94 (56.17)	65.38 (53.98)	72.67
T_3	*Azadirachta indica*	10	54.54 (47.58)	45.84 (42.58)	41.16 (39.88)	40.93 (39.72)	45.61
T_4	*Zingiber officinale*	10	23.69 (29.10)	23.65 (29.07)	24.89 (29.89)	25.78 (30.41)	24.50
T_5	*Parthenium hysterophorus*	10	68.78 (56.05)	58.60 (49.97)	53.24 (46.85)	51.00 (45.56)	57.90
T_6	*Citrus aurantifolia*	10	15.95 (23.45)	18.35 (25.12)	21.05 (27.16)	23.35 (28.72)	19.67
T_7	*Curcuma longa*	10	39.06 (38.63)	34.02 (35.56)	36.86 (37.28)	37.37 (37.63)	36.82
T_8	*Ocimum sanctum*	10	61.52 (51.67)	50.29 (45.14)	50.82 (45.44)	46.97 (43.24)	52.40
T_9	*Lantana camara*	10	30.30 (33.38)	29.87 (33.06)	29.72 (33.00)	29.68 (32.96)	29.82
T_{10}	Control	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
SE(m)±		-	1.57	1.97	2.01	2.05	-
CD (P=0.05)		-	4.68	5.86	5.98	6.11	-

*Figures in parentheses are angular transformed values

antifungal activity against all the test fungi has been shown by Limonoids 2, 3, 6, and 8. It is also reported that *Sclerotium rolfsii* was highly sensitive to these four limonoids A sesquiterpene lactone compound Parthenin is isolated from *Parthenium hysterophorus* which reduced germination of fungal spores viz., *Pestalotia* sp., *Cladosporium herbarum* and *Helminthosporium sativum*. It causes lobulations, hyphal wall thickening and restricted mycelia growth in *Curvularia lunata* (Girija, 1993).

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