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EVALUATION OF BOTANICAL EXTRACTS AGAINST *XANTHOMONAS AXONOPODIS* PV. *CYAMOPSIDIS*

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SUMMARY

India is the major producer of cluster bean (*Cyamopsis tetragonoloba* L. Taub.) contributing about 82 per cent of the total production of the world and bacterial leaf blight is one of its most destructive disease caused by *Xanthomonas axonopodis* pv. *cyamopsidis*. It causes yield loss of 50–70 per cent of cluster bean under severe conditions, if appropriate measures to combat the disease are not taken timely. The disease effects crop productivity in all growing regions of the world. In Haryana state, the bacterial blight has been recognized as most important limiting factor in reducing the yield. It is both seed borne and air borne in nature and affects all above ground parts of plant. Chemicals have played significant role in management of the disease but their use in excessive amount led to detrimental effect on the ecosystem such as residual toxicity, harmful effect on living beings and the whole environment, especially on the beneficial predators, parasites and microorganisms. Keeping this in view, the present investigation has been devised on disease management using various biorational components during 2020-2021 at Hisar. It was observed that *Azadirachta indica* was found most effective with 18.04 per cent growth inhibition followed by *Zingiber officinale* under *in vitro* conditions. Under screen house conditions, minimum per cent disease incidence (PDI) of 26.67 per cent was observed in treatment of *Azadirachta indica* @ 25 per cent w/v followed by treatments of *Zingiber officinale* @ 25 per cent w/v.

Key words: Bio-agents, botanicals, growth inhibition, *Xanthomonas axonopodis* pv. *cyamopsidis,* bacterial blight, *Azadirachta indica*

Cluster bean (*Cyamopsis tetragonoloba* L. Taub.) (2n=14) is an indigenous, self-pollinated, drought tolerant legume crop which belongs to the family Fabaceae. In India, it is commonly known as guar and poor man's crop. It is grown in semi-arid and arid regions as a *Kharif* season crop. Primarily, it is a deep rooted and drought hardy summer annual legume which is grown mainly in the drier habitats. Although, cluster bean is well-known for remarkably higher adaptative measures towards scanty and erratic rainfall and possess less inputs requirement and soil enrichment properties but it is a photosensitive crop and requires specific climatic condition to grow. For proper germination of seed, 20-25°C soil temperature isideal, crop requires long day period for vegetative growth and short day period for floral development and pod formation. Being a drought tolerant crop, it performs well under wide range of rainfall (300-550 mm), but heavy rainfall encourages many diseases. Green and tender pods of cluster bean are used as a vegetable in many parts of the country. It is also grown as a forage crop and used for reclamation of saline and alkaline soils (Ghorpade *et al.,* 2018). This crop has attained a status of industrial importance in recent years, mainly due to the presence of gum in its endosperm, which constitutes about 30-32 per cent of the whole seed. Cluster bean seed is used as a concentrate for livestock and poultry. Its multiple uses make it an important component of the cropping systems. This crop has recently gained the status of industrial crop due to the high galactomannan content in the endosperm of its seed which has multiple industrial applications and thus becomes a major foreign exchange earner for the area. Cluster bean gum derivatives that are commercially important include hydroxy and carboxy guar gum, oxydized guar gum, acetate guar gum, guar gum formate, sulphated guar gum, guar gum acryl amide and carboxyl methyl hydroxyl propyl guar gum. Its derivatives are used in various industries like textile, paper, cosmetics, mining, petroleum, pharmaceutical, food processing, oil drilling and explosives (Bharti *et al.,* 2020).

India is one of the main producers of cluster bean accounting for about 82 per cent of the total production of the world because of favourable climatic conditions for the crop production and grown in the north-western states of India under rainfed conditions, namely, Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh and Madhya Pradesh (Bagri *et al.,* 2021). In India, area under cluster bean during 2019-20 was 3.14 mha with a production and productivity of 1.52 million tonne and 484 kg ha^{-1} (Anonymous, 2020). Rajasthan is the largest producing state of cluster bean followed by Haryana. Share of Haryana in cluster bean production in India varies from 18 to 30 per cent in different years. The districts in Haryana involved in the production of cluster bean are Bhiwani, Dadri, Gurgaon, Mahendragrh, Rewari and Hisar. After seeing greater avenues with the crop during previous years in Rajasthan and Haryana, farmers of Andhra Pradesh have also started the cultivation of this crop for seeds in more than 1000 ha (Agarwal *et al.,* 2019).

Bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*), alternaria leaf spot (*Alternaria cyamopsidis*), anthracnose (*Colletotrichum capsici* f.sp. *cyamopsidis*), charcoal rot/damping off (*Macrophomina phaseolina*), dry root rot/ leaf blight (*Fusarium solani* and *Rhizoctonia solani*) and wilt (*Fusarium caeruleum*) are important diseases of arid legume clusterbean (Kumhar *et al.,* 2018).

Among all these diseases, bacterial leaf blight is one of the most destructive disease of clusterbean caused by *Xanthomonas axonopodis* pv. *cyamopsidis* and confines clusterbean productivity in all growing regions especially in irrigated and dry uplands where predisposing factors favour disease development to epidemic proportions. The pathogen is seed-borne in nature and provides primary inoculums for secondary spread. During the monsoon, scattered rains, high humidity, cloudy weather, and warm temperature (28-30°C) favours the development of bacterial leaf blight and later, infection from blighted leaves spreads to stem via petiole and stem cracks may develop in advanced stage of infection. The pods are also heavily spotted. Early infection may reduce yield more severely (Amin *et al.,* 2017). In general, this bacterial infection is controlled mainly through application of anti-bacterial chemicals, adoption of less susceptible or resistant cultivars and destruction of the diseased plant material. Biological control is an ecological-friendly approach which is less toxic for non-target species and has potential to suppress the growth of antagonistic pathogens (Macha *et al.,* 2021). Seed treatments play very important role in managing bacterial blight, therefore need scheming of effective disease management strategies in order to minimize the disease to threshold level and maximize the crop production. Hence, regular monitoring of persistence and distribution of this pathogen is required and planned to manage the bacterial leaf blight (Bagri *et al*., 2021).

Excessive use of chemicals in crop production systems has detrimental effects on the ecosystem such as residual toxicity, harmful effects on living beings and the whole environment, especially on the beneficial predators, parasites and microorganisms (Sunil *et.al*., 2003). Many plant and microbial species possess natural substances that are toxic to many pathogens, also provides eco-friendly and economical management of this disease. Plant extracts of *Alliums ativum*, *Allium cepa*, *Azadirachta indica*, *Capsicum annum*, *Calotropis gigantea*, *Pongamia pinnata*, *Dalbergia sissoo*, *Eucalyptus* sp., *Melia azedarach* and *Zingiber officinalis* were found effective against many plant pathogens. The medicinal and anti-microbial properties of plants and their products have been known since the ancient times. These are not only biologically active but easily biodegradable and relatively safe to nature (Naqvi *et al.,* 2018).

Researchers have used various traditional disease management strategies as control measures so far but not much has been done in the evaluating the antagonistic nature of botanicals against bacterial leaf blight in guar. Therefore, objectives of the research is to find out efficacy of bio-rational components against *X. axonopodis* pv. *cyamopsidis in vitro* conditions.

MATERIALS AND METHODS

Preparation of botanicals/plant extracts

For the preparation of plant extract, the fresh leaves or other plant parts of respective plants were collected and washed thoroughly with distilled water to remove soil, dirt and microbial spores. Then plant material was macerated in appropriate volume of water in blender (1:1 w/v). The macerated material was squeezed properly using double-fold muslin cloth and filtered through Whatman filter paper No.1 to get aqueous extract. The aqueous extracts collected were subjected to filter sterilization and centrifuged at 4000-6000 rpm for about 20 minutes until clear supernatant was obtained through millipore filter attached to a glass syringe (5 ml). Obtained filtrate was collected in sterile

screw cap vials for further use and served as 100 per cent concentration crude plant extract.

In vitro evaluation of botanicals components against *Xanthomonas axonopodis* pv. *cyamopsidis*

The aqueous extract of different plant species *viz. Eucalyptus globolus* leaves, *Azadirachta indica* leaves, ginger rhizome *(Zingiber officinale*), turmeric powder (*Curcuma longa*) and *Pongamia pinnata* leaves, were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis* using paper disc method (Mayer, 1962). Different concentrations (0, 5, 10, 15, 20 and 25) per cent (w/v) of botanical extract solutions were created. The 5mm-diameter filter paper discs were placed on the medium of the plates after being soaked in the appropriate botanical extract solutions. To allow the diffusion of chemicals into the medium, the infected plates were stored in the refrigerator at 5 °C. After that, the plates were incubated at 27°C while the generation of inhibition was monitored 48 to 72 hours following the inoculation, observations regarding the botanical inhibitory zone were noted. These tests were replicated four times. The percent growth inhibition in each treatment was calculated according the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition

C = Diameter of pathogen colony in control
T = Diameter of pathogen colony in treatment

Evaluation of efficacy of selected botanical extracts against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions

Cluster bean crop was raised in the earthen pots containing sandy-loam soil mixed with well-decomposed FYM in the screen house of Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Susceptible variety (Pusa Navbahar) of clusterbean was sown in pots on 30th July, 2021 at uniform depth and distance to get optimum plant stand in pot. Rouging was done to remove weeds. Plants were irrigated using tap water as and when required. The pots containing 25 to 30 days old plants were artificially inoculated with *Xanthomonas axonopodis* pv. *cyamopsidis* by spraying the bacterial suspension of 1×10^8 cfu ml^{-1}. The pots were covered with the polythene bags for next 48 hours to maintain humidity. After 6 to 8 days of inoculation, when characteristic blight symptoms were shown on plants, botanicals and bio-agents found effective in inhibiting bacterial blight under *in-vitro* condition were sprayed over the crop plants. The suspension of various concentrations was prepared in sterilized water just before inoculation. Total number of replications were three.

TABLE 1
Treatments under screen house conditions

S. No.	Treatments
1.	*Eucalyptus globulus* @ 25% w/v
2.	*Zingiber officinale* @ 25% w/v
3.	*Azadirachta indica* @ 25% w/v
4.	*Curcuma longa* @ 25% w/v
5.	*Pongamia pinnata* @ 25% w/v
6.	Control

Observation on per cent disease incidence was recorded. Disease incidence was determined as number of plants affected per pot and expressed in percentage.

Disease incidence was calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

In-vitro evaluation of botanicals against *Xanthomonas axonopodis* pv. *cyamopsidis*

The results of the experiment depicted that *Azadirachta indica* proved to the most effective botanical against *X. axonopodis* pv. *cyamopsidis* (18.04%) growth inhibition followed by *Zingiber officinale* (15.75%) and *Eucalyptus globulus*. (13.15%) and minimum was recorded with *Pongamia pinnata* (8.10%).

Azadirachta indica reported significantly higher zone inhibition of 18.04 per cent at 25 per cent concentration followed by 20 per cent (17.01%) and15 per cent (16.14%). *Azadirachta indica* was found highly effective as compared to other tested botanicals even at low concentration, as it showed higher growth inhibition (15.89%) at 10 per cent concentration which was statistically at par with *Zingiber officinale* at 25

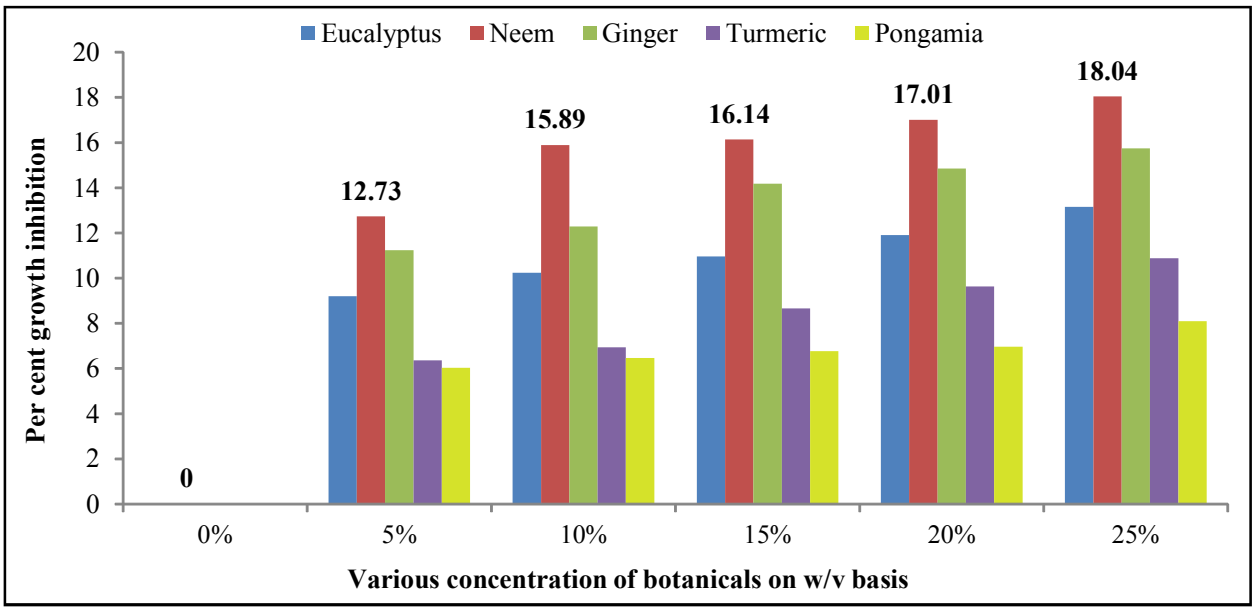

Fig. 1. *In vitro* evaluation of botanical extracts against *Xanthomonas axonopodis* pv. *cyamopsidis*.

per cent conc. (15.75% zone inhibition) followed by *Zingiber officinale* at 20 and 15 per cent and *Eucalyptus globulus* @ 25 per cent concentration on w/v basis with 14.86, 14.18 and 13.15 per cent zone inhibition, respectively. Among the concentrations, 15 and 20 per cent fairly good per cent growth inhibition was obtained but 25 per cent was proved the best and show significantly higher inhibition percentage of various botanicals.

Zingiber officinale was also found effective next to *Azadirachta indica* against the test pathogen and recorded 15.75, 14.86 and 14.18 per cent growth inhibition at 25, 20 and 15 per cent concentration on w/v basis. At low concentration of 5 and 10 per cent, both *Curcuma longa and Pongamia pinnata* proved least effective. The results were in agreement with others (Negi *et al.* 2015).They observed that neem leaf extract significantly inhibited the bacterial growth, followed by vilyati babool and babool leaves and had an inhibition zone of 3.20 mm at 10 per cent concentration. Similar results were reported that neem products (plantolyte and agricare) were found effective in concert with

TABLE 2
In-vitro evaluation of botanicals against *Xanthomonas axonopodis* pv. *cyamopsidis*

Treatments	Per cent zone inhibition at various concentrations (%)					Mean
	5	10	15	20	25	
Eucalyptus globulus	9.20 (17.64)	10.24 (18.65)	10.96 (19.32)	11.91 (20.17)	13.15 (21.25)	9.24 (16.27)
Azadirachta indica	12.73 (20.89)	15.89 (23.48)	16.14 (23.67)	17.01 (24.34)	18.04 (25.12)	13.31 (19.68)
Zingiber officinale	11.24 (19.57)	12.29 (20.51)	14.18 (22.11)	14.86 (22.65)	15.75 (23.37)	11.39 (18.13)
Curcuma longa	6.36 (14.58)	6.94 (15.20)	8.66 (17.09)	9.64 (18.06)	10.88 (19.23)	7.08 (14.12)
Pongamia pinnata	6.03 (14.21)	6.46 (14.72)	6.77 (15.07)	6.97 (15.30)	8.10 (16.51)	5.72 (12.73)
Mean	9.11 (17.38)	10.37 (18.51)	11.34 (19.45)	12.08 (20.10)	13.18 (21.10)	
	Treatments		Concentration		Treatments x Concentration	
S. Em±	0.132		0.145		0.324	
C. D. (P=0.05)	0.373		0.408		0.912	

Figures given in parenthesis represent angular transformed value.

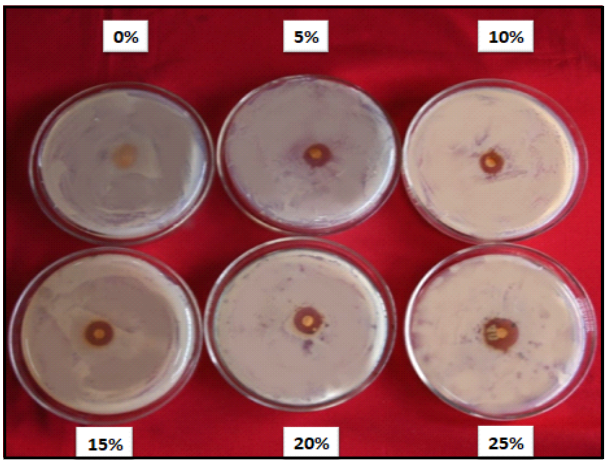

Plate 1. Percent zone inhibition by *Azadirachta indica* at various concentrations.

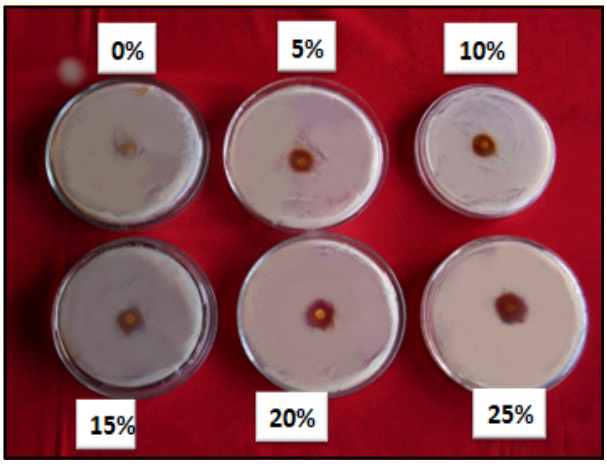

Plate 2. Percent zone inhibition by *Eucalyptus globulus* at various concentrations.

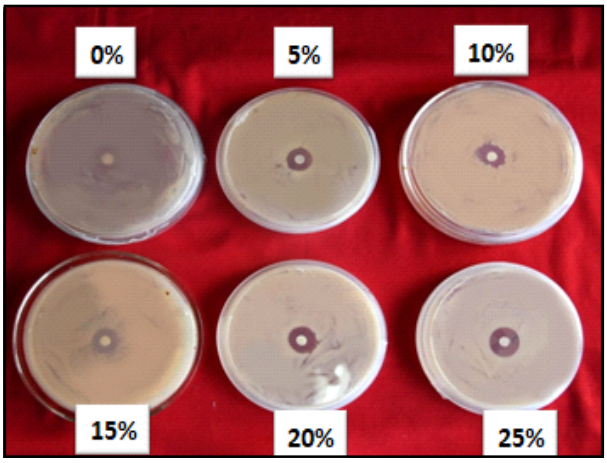

Plate 3. Percent zone inhibition by *Zingiber officinale* at various concentrations.

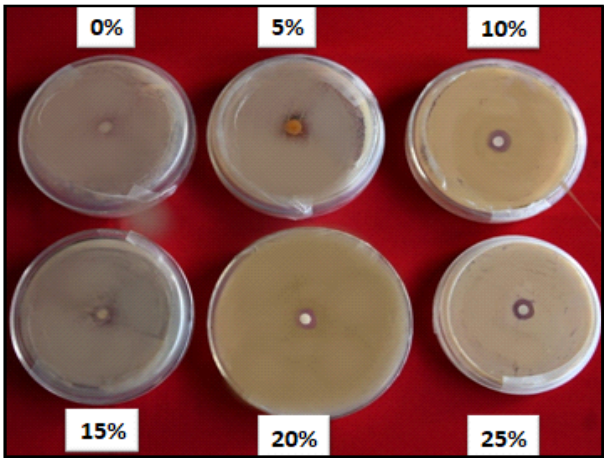

Plate 4. Percent zone inhibition by *Curcuma longa* at various concentrations.

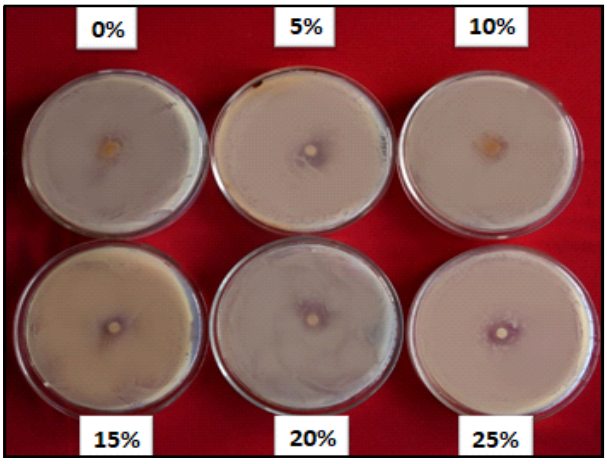

Plate 5. Percent zone inhibition by *Pongamia pinnata* at various concentrations.

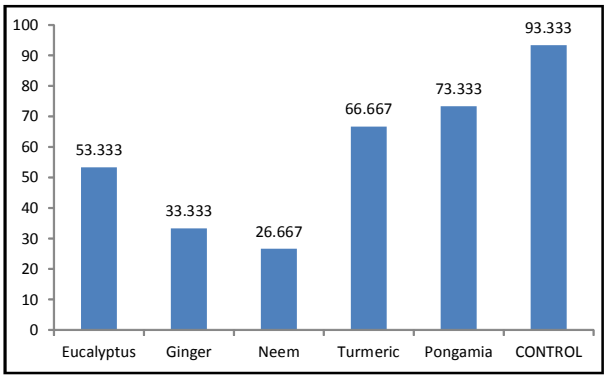

Fig. 2. Efficacy of potential botanical extracts concentration against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions.

antibiotics and fungicides (Raju *et al.,* 2012; Hulloli *et al.,* 1998). Neem formulations stipulated the efficacy of antibiotics and fungicides in synergistic manner. Ethyl acetate and water extract of *Azadirachta indica* inhibits the *X.axonopodis* pv. *cyamopsidis in vitro* (Antre *et al.*, 2016; Kishanawat *et al.,* 2021).

Among various plant plant oils such as neem, mahua, thyme, clove, eucalyptus, lemongrass, peppermint, citronella found to inhibit the growth of the *Xanthomonas axonopodis* pv. *Punicae* (Chowdappa

TABLE 3
Tests of efficacy of potential botanical extracts against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions

Treatments	Disease incidence (%)	Disease reduction (%)
Eucalyptus globulus @ 25% w/v	53.33 (46.90)	42.86
Zingiber officinale @ 25% w/v	33.33 (35.00)	64.28
Azadirachta indica @ 25% w/v	26.67 (30.78)	71.43
Curcuma longa @ 25% w/v	66.67 (54.97)	28.57
Pongamia pinnata @ 25% w/v	73.33 (59.19)	21.43
Control	93.33 (81.14)	0.00
C. D. (P=0.05)	16.31	-
S. Em±	5.23	-
C.V.	17.67	-

et al., 2018). Observations were made that neem and garlic @ 20 per cent concentration, displayed the largest zone of inhibition, measuring 1.73 cm and 1.67 cm, respectively followed by guava (1.57 cm) and castor (1.43 cm), with *turmeric* having the inhibition zone of 1.13 cm against *Xanthomanas axonopodis* pv. *citri* (Kanwar *et al.*, 2016). The results of the laboratory experiment findings advocated the fact that neem had valuable antimicrobial properties (Giri *et al.*, 2008; Gena *et.al.*, 2008).

Tests of efficacy of selected botanical extracts against *Xanthomonas axonopodis* pv. *cyamopsidis* under screen house conditions

The results presented in the Table 3 and Fig 2 indicated under screen house conditions, minimum per cent disease incidence (PDI) of 26.67% was observed in treatment of *Azadirachta indica* @ 25% w/v followed by *Zingiber officinale* @ 25% w/v with PDI of 33.33 as compared to control where the PDI was maximum (93.33 per cent). *Eucalyptus globulus*, and *Curcuma longa* reduced the disease by 42.86 and 28.57 per cent, respectively. Among the treatments, the lowest disease reduction and highest PDI was observed in treatment *Pongamia pinnata.* Similar results were recorded which reported that maximum disease severity reduction of bacterial blight was obtained with seed treatment from various botanical extracts *viz.* garlic, *Azadirachta indica*, *Calotropis* sp. which provided satisfactory control of the disease and protected the crop from the disease upto 35 days after sowing [14]. Three sprays of neem seed extract (5.00%) were determined to be the most effective among the botanicals because they resulted in the lowest disease intensity (14.92 %) and the highest level of disease control (23.91 %) (Giri *et al.,* 2008).

CONCLUSION

Efficacy of botanicals *w*as tested for per cent zone inhibition of *Xanthomonas axonopodis* pv. *cyamopsidis* under *in-vitro* conditions. *Azadirachta indica* was found most effective botanical against *Xanthomonas axonopodis* pv. *cyamopsidis* with 18.04% followed by *Zingiber officinale* (15.75%) and *Eucalyptus globulus*. (13.15%) and minimum was recorded with *Pongamia pinnata* (8.10%). Aqueous extract of *Azadirachta indica* had significant superiority over other treatments of botanicals at various concentrations with highest inhibition of 18.04% at 25% concentration followed by *Azadirachta indica* at 20% (17.01%) and *Azadirachta indica* at 15% (16.14%). *Pongamia pinnata* was found least effective among all treatments, as it inhibited only 8.10, 6.97, 6.77, 6.46 and 6.03 per cent growth inhibition at 25, 20, 15, 10 and 5 per cent concentration on w/v basis, respectively.

Under screen house conditions results indicated that minimum per cent disease incidence (PDI) of 26.67 per cent was observed in treatment of *Azadirachta indica* @ 25 per cent w/v followed by treatments of *Zingiber officinale* @ 25 per cent w/v with PDI of 33.33 as compared to control where the PDI was maximum (93.33%) under screen house conditions. The outcome of the study firmly conclude that the use of various botanical formulations can perform very well for the management of blight disease in cluster bean.

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