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In Vitro Assay of Antifungal Activity of Various Elicitors and Binders against *Curvularia lunata*

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Abstract

In the present study *in vitro* antifungal activity of various elicitors and binders were evaluated against *Curvularia lunata* caused leaf spot disease maize. Antifungal activities were assayed by poison food technique. Among the all Elicitors and all binders best optimum activity was observed for clove bud oil cake and cow dung i.e. 51% and 33.88% respectively against *Curvularia lunata*. On the basis of results obtained, best active elicitor i.e. clove bud oil cake and binder. Cow dung can be used to develop the plant extract based bio-formulation for effective control of leaf spot disease of maize in an eco-friendly manner.

Keywords: Elicitors; Binders; Plant Extract; Bio-Formulation

Introduction

Plants are exposed to an array of biotic stresses like bacterial, fungal and viral infections. This leads to an enormous loss to plant yield. There are different options available for the farmers to prevent their crop from disease. Some options include development of resistant cultivars, crop rotation, tillage, biological control, and chemical pesticides. Nearly all chemical fungicides or pesticides have a direct antibiotic principle. But their use at commercial level on a large scale is too expensive; application is cumbersome and has been proved to be carcinogenic. Therefore, significant efforts have been made to develop environmental-friendly strategies for management of plant diseases [1,2].

In order to develop a new active bio-formulation as biological agent for control of plant disease screening is done to find out an active elicitor and binder which enhance the efficacy of plant extract. Initially the term elicitor was used for molecules that are capable of inducing the production of phytoalexins, but it is now normally used for any compounds that stimulate any kind of plant defense [3-8]. Elicitors include both substances originating from pathogen i.e. exogenous elicitors and compounds generated from plants, i.e. endogenous elicitors [9]. Elicitors are also classified as biotic or abiotic, physical or chemical, and complex or depending on their origin and molecular structure.

Several agro-based ravage and byproducts are known which play an important role in the management of crop plant diseases. They act directly or indirectly on plant pathogens and inhibit the growth and multiplication of the pathogen or induce resistance in crop. Some reports are available on use of agro based ravage and byproduct for control plant diseases like oil cakes [10-13].

Binder is a material which holds other materials together (elicitors and extracts) to form a cohesive formulation. Several materials such as guar gum, gum acacia etc. are used as binders. Guar gum, also known as guaran, is the ground endosperm of guar beans. In recent year researches have used guar gum as a rate controlling mediator for controlled delivery of bioactive molecules [14-17]. Gum acacia is used in traditional medicine and pharmaceutical industry but no

reports of it are available for controlling plant disease as use as binder [18-20].

The Hindu Vedas say that the cow is blessed and should be worshiped. In India, cows are very important living thing resources and are highly useful in agricultural and dairy industry [21]. Panchagavya is a term used to illustrate five major substances, obtained from cow, which include cow's, milk, curd, ghee, urine and dung. All the five products possess medicinal properties against many diseases. This kind of healing is called Panchagavya therapy [22]. Rajeswari, et al. [23] investigated the significant antifungal activity of cow dung extract against *Escherichia coli* and *Klebsiella pneumonia.* Krunal, et al. [24] also illustrated the significant activity of cow urine and cow dung against phytopathogenic fungus *Colletrotrichum falcatum.* In the present study, selection of suitable binder and elicitor to get optimum antimicrobial efficiency from plant extract.

Materials and Methods

In the present study, antifungal activity of various elicitors and binders against *Curvularia lunata* was studied. For the *in vitro* antifungal activity there are seven type of elicitors i.e. ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake ,clove bud oil cake, coconut oil cake, and neem oil cake and three type of binders i.e. guar gum, gum acacia and cow dung were used.

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There are seven types of elicitors i.e. ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, clove bud oil cake, coconut oil cake, neem oil cake, etc. and three types of binder i.e. guar gum, gum acacia and cow dung have been used to study the antifungal activity against *Curvularia lunata*. 20 gm of each elicitor was dissolved in 100 ml of autoclaved water for 24 h. The mixture was then filtered and the filtrate was further used for antifungal activity. The antifungal activity of each elicitor was tested using poison food technique. 1 ml of each elicitor with 9 ml molten sterile PDA culture medium was poured into pre-sterilized petri-plates (9 cm diameters) and allowed to solidify at room temperature. Thus prepared petri-plates were inoculated aseptically with 6mm disc of test pathogen's cultures which was placed at the centre of the plate. The Petri-plates were then incubated at 28 ± 2ºC for seven days, only PDA culture media are used as control series. Antifungal activity of each elicitor was measured as a function of increase in growth diameter of 6 mm disc of inoculums.

$$\text{Mycelial growth inhibition} = \frac{gc\text{-}gt}{gc} \times 100$$

gc = growth of fungal colony after 7 days incubation period in control set subtracting the diameter of inoculums disc.
gt = growth of fungal colony after 7 days incubation period in treatment set subtracting the diameter of inoculums disc.

Results and Observations

The results of antifungal activity of various elicitors against *Curvularia lunata* are given in Table 1 and Figure 1A. Among the all elicitors clove bud oil cake showed best activity i.e. 51.00% followed by neem oil cake i.e. 0.11% and the results of antifungal activity of various binders against *Curvularia lunata* are given in Table 2 and Figure 1B. Among the all binders the best activity was observed with cow dung i.e. 33.88% followed by guar gum and gum acacia i.e. 0.04% and 0.02%, respectively.

S. No.	Elicitors	Growth diameter after 7 days (mm) ± SD	Percentage (%) mycelial growth inhibition
1.	Neem oil cake	73.33 ± 0.57	0.11
2.	Mustard oil cake	81.66 ± 0.57	0.012
3.	Cotton oil cake	75.66 ± 1.15	0.08
4.	Sesame oil cake	77.66 ± 0.57	0.06
5.	Clove bud oil cake	40.33 ± 0.57	51.21
6.	Ground nut oil cake	80.66 ± 0.57	0.02
7.	Coconut oil cake	81.33 ± 0.57	0.016
8.	Control	82.67 ± 0.57	-

Table 1: Antifungal activity of various elicitors against *Curvularia lunata.*

S. No.	Binders	Growth diameter after 7 days (mm) ± SD	Percentage (%) mycelial growth inhibition
1.	Cow dung	54.66 ± 0.57	33.88
2.	Guar gum	79.33 ± 0.57	0.04
3.	Gum acacia	81.00 ± 1.00	0.02
4.	Control	82.67 ± 0.57	-

Table 2: Antifungal activity of various binders against *Curvularia lunata.*

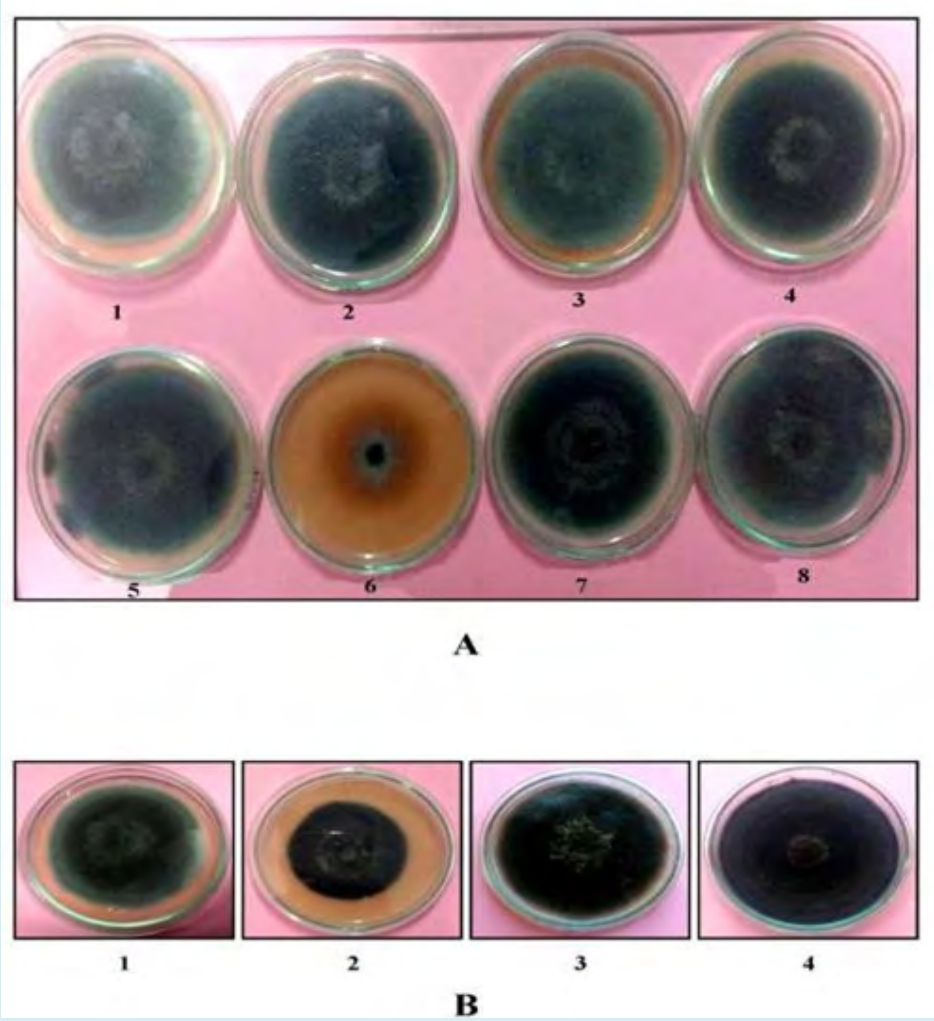

Figure 1: Antifungal activity of elicitors and binders.

(A) Elicitors (1) Cotton oil cake, (2) Mustard oil cake, (3) Neem oil cake, (4) Sesame oil cake, (5) Ground nut oil cake, (6) Clove bud oil cake, (7) Coconut oil cake, and (8) Control (Only PDA media).

(B) Binders (1) Guar gum, (2) Cow dung, (3) Gum *Acacia*, and (4) Control (Only PDA media).

Discussion

The use of elicitors and binders in crop plant protection and pest management is use as a new control method [25] and thus, the current experiences come from experimental trial, and not yet from large scale agricultural use. Exploiting the used of elicitor and binder to control plant disease is because of the reduced environment hazards and reduced damage from pathogens. Only plant extract used in controlling plant disease is very expensive and a lot of plant

parts are required for obtaining it. Use of elicitor and binders in combination with plant extracts is gaining importance because it very cheap and has long term positive effect [26-28]. In this study, total seven elicitors, i.e. ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, clove bud oil cake, coconut oil cake, neem oil cake, etc. and three binders, i.e. guar gum, gum acacia, and cow dung, were used to evaluate the antifungal activity against test pathogen. Many researchers have evaluated the antimicrobial activity of elicitors and binders against different pathogens and use of elicitor for control of plant diseases is ecofriendly and very effective. Harish, et al. [29] screened four oil cakes i.e. mahua cake extract, neem cake extract, castor and gingelly cake extracts against *Bipolaris oryzae*, the causal agent of brown spot disease in rice and amongst the all cake extracts neem cake extract showed the maximum inhibition of mycelial growth and spore germination followed by mahua cake extract, castor and gingelly cake extract. Coventry & Allan [30] illustrated the antimicrobial effect of neem seed extract against different plant pathogens. Singh, et al. [31] reported that foliar spray of aqueous extract of neem cake showed significant antifungal potential against powdery mildew of balsam.

The antimicrobial properties of gossypol from cotton plant have been reported by several researchers [32-37]. Coconut is an important fruit tree in the world the various product of coconut include coconut water, copra, raw kernel, coconut cake, coconut oil, are very useful with many kind of properties such as antibacterial, antifungal, antioxidant, immunostimulant, hepatoprotective and also contain microminerals and nutrients [38] and antimicrobial potential of ground nut oil cake, mustard oil cake, sesame oil cake, clove bud oil cake and oil has been studied [39-48].

In case of *Curvularia lunata* significant inhibitory activity was observed with only clove bud oil cake followed by neem oil cake. Other elicitors did not show significant antifungal activity. The reason for this may be presence of specific secondary metabolites in clove which effect the growth and metabolism of the test fungus. Clove bud oil cake contain eugenol, eugenyl acetate and caryophyllene which is known for the antifungal activity [46,49-50]. As compare to this other elicitors do not contain these secondary metabolites or the amount of these secondary metabolites is not enough to inhibit the growth of test fungus. This proves that growth inhibitions by secondary metabolites is very specific and probably depend on the ligand binding with surface protein of the test fungus [51-53].

The next step in the process of exploring a suitable binder for enhancing the antimicrobial activity of plant extract into culture medium the poison food technique was used. Three binders namely guar gum, gum acacia and cow dung, etc. used to test the antifungal activity against test pathogen. All of these binders have antimicrobial activity against different pathogens. Guar gum (galactomannan polysaccharide) obtains from Guar, *Cyamopsis tetragonoloba* L. or cluster bean is a drought tolerant annual legume and also contains 13% dry matter crude saponin [54]. Hassan, et al. [55] reported the significant antibacterial activity of saponin rich guar gum against *Escherichia coli, Staphylococcus aureus* and *Salmonella Typhimurium.* Hada & Sharma [11] also evaluated the antifungal activity of guar gum, gum acacia and cow dung against *Alternaria solani* and best antifungal activity was observed with cow dung followed by gum acacia, guar gum. Gum acacia (galactoaraban) is obtained from tree plant *Acacia nilotica* Linn. All part of the plant i.e. gum, stem bark, leaves and fruits shows medicinal properties [56-58]. Cow dung posse's antimicrobial properties against different pathogens [59]. Here, screened out of all the binders, cow dung possesses significant antifungal activity against *Curvularia lunata* [60-67].

Conclusion

Results of current study indicated that best active elicitors i.e. clove bud oil cake and binder i.e. cow dung can be used to develop the plant extract based bio-formulation for effective control of leaf spot disease of maize in an eco-friendly manner. These bio-formulations could be considerable in the direction of sustainable agriculture without destroying biological system. The manufactured bio-formulations have vast potential to be economically explored for agriculture use.

Author's Contributions

TSB: Planned and designed the experimental work, performed the experiments, wrote the manuscript. MM: Analyzed all the data, results assessment and evaluation, implemented all the statistical analysis, prepared and wrote the final version of the manuscript. KS: Supervised the entire work. The final version of the manuscript read and approved by all the authors.

Conflict of Interest

The authors declare that there is no conflict of interest.

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