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ANTIFUNGAL EFFICACY OF *CAESALPINIA BONDUCELLA* (L.) FLEMING AGAINST PHYTOPATHOGENIC FUNGUS MACROPHOMINA PHASEOLINA (TASSI) GOLD

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Abstract

Caesalpinia bonducella (L.) fleming is valuable herb remedial plant. The current work was conducted to evaluate the antifungal properties of *C. bonducella In vitr*o and *In vivo* against *Macrophomina phaseolina* (tassi) gold by food poison technique. *In vitr*o, antifungal properties of the leaves extract of *C. bonducella* in different solvents *viz.* aqueous, ethanol, and hexane at concentrations of 5, 10, 15, and 20% was analysed. The data disclose that an increased concentration of leaves extract suppressed pathogen growth. Among all the solvents, the highest antifungal activity was obtained in an aqueous extract at 20% concentration followed by ethanol extract at 15 and 20%. *In vivo*, to evaluate the antifungal activity of *C. bonducella,* pot experiments were performed. The obtained data showed that aqueous extract of *C. bonducella* leaves significantly lowered the colonization% of *M. phaseolina* in artificially infested seeds of the castor plant (*Ricinus communis* L.) and promoted plant growth as compared to the control. The finding of the present studies evaluated that, the antifungal efficacy of *C. bonducella* was effective in the management of the phytopathogenic fungus *M. phaseolina* in both *In vitr*o and *In vivo* conditions.

Key words: Solvents, *Ricinus communis* L, Food poison technique, Antifungal activity.

Introduction

In the recent era, plant health faces threats from pathogenic microbes globally. Chemicals are a vital source of crop protection for farmers for increasing agriculture productivity over the past few decades. Nevertheless, due to the vast usage of chemicals, pathogens show resistance to antimicrobial agents (De Weger *et al*., 1995). Nowadays, biological control is concerned with food safety and a healthy environment. To decrease the utilization of agrochemicals and the development of sustainable agriculture biological control reflects the desired output in many sustainable plant diseases (Hyakumachi, 2013). *Macrophomina phaseolina* is a cosmopolitan soil-borne fungus that infects about 500 types of plant species (Dhingra & Sinclair, 1978). Diseases like charcoal rot, seedling blight, and stem and root rot were reported due to *Macrophomina phaseolina* (Ghosh *et al*., 2018).

Plant-based antifungal products are considered positive for the management of the fungal population. The metabolites derived from these plants reduced mycelium growth with no harmful side effects and are eco-friendly as well (Singh *et al*., 2006; Shrestha and Tiwari, 2009). Previous literature emphasized the plants inhibitory impact and their potential usage for the management of plant disease (Farooq *et al*., 2010; Ortiz *et al*., 2010; Gupta & Tripathi, 2011).

Caesalpinia bonducella (L.) fleming is belong to the Caesalpiniaceae family. Generally, it is known as Fever Nut, Bonduc Nut also (Khare, 2008). *C. bonducella* can grow about 6m long, a shrub that can climb on different plants. Curved spines are found on the stem and their seeds are grey known as nicker nut which is 2cm in length. Seeds are dispersed through ocean currents (Sasidharan *et al*., 2021).

This medicinal plant is mostly found in the different regions of Asia and Africa having humid and hot climates (Gupta *et al*., 2003). This medicinal plant was commonly seen in continents of Asia like Indonesia, India, and Sri Lanka (Woerdenbag & Kayse, 2014). Six species of *C. bonducella* were also reported in Pakistan (Khatun, 2006). Genus *Caesalpinia* includes more than 500 divers having many pharmacological properties (Zanin *et al*., 2012). The plant was described under the same name as C. *bonducella* (Syn. *C. bonduc)* which caused a lot of confusion (Moon *et al*., 2010; Rastogi *et al*., 1990). The species, for example, *C. nuga* (Kapoor, 2000; Jabbar, 2007; Asolkar, 1992; Kirtikar & Basu, 1975) and *C. jayoba* are synonyms for *C. crista*. *C. pjayoba* is a species oriented from the Arabic word "Bonduce" which means a little ball it specifies the globe form of its seeds (Konan *et al*., 2014).

In Indian tradition, *C. bonducella* is commonly known as antiperiodic, calming, antipyretic, and anthelmintic, moreover, it is used as an herbal remedy in case of spasms, orchitis, leprosy, skin ailments, paralysis, and nervous grumbles. In addition, it is also found to have an anti-aging, anti-cancer, and antibacterial effect (Ali *et al*., 2009). It is a vital plant as an herb, plant root, bark, seed, and leaves tend to treat different colic fever, malaria, pneumonia, swelling, pulmonary tuberculosis, menstruation cycle, and edema (Komal *et al*., 2010).

The potential of *C. bonducella* has been observed against *Aspergillus niger, Candida albicans, Fusarium oxysporum,* and *Alternaria solani* (Shukla *et al*., 2011). The presence of phytochemicals like steroids, glycosides, tannins, alkaloids, phenols, resins, flavonoids, and saponins was reported as antifungal agents in *Caesalpinia bonducella* (Shukla *et al*., 2011; Irkin & Korukluoglu, 2009; Zhang *et al*., 2006). (Gupta *et al*., 2004) also observed the antifungal and antibacterial capability of *Caesalpinia bonducella* in methanolic extracts. The antifungal activity of *C. bonducella* is due to high flavonoid, phenolic, and steroid contents which exert the biological impact by doing ROS and antioxidant activity (Peter *et al*., 1997; DeFeudis *et al*., 2003; Takeoka & Dau, 2003). The literature on the antifungal efficacy of *C. bonducella* was reported by different researchers.

Material and Methods

Collection of plant material: *C. bonducella* (Family Caesalpiniaceae) leaves were collected from the University of Karachi. Leaves were washed and shade dried then ground into powder finely then stored in airlocked containers for further use.

Preparation of plant extract: Soxhlet apparatus used for the composing of concentrations from selected plants. The amorphous form of plant material (20 g) was extracted in the soxhlet apparatus for 2 to 5 hours in ethanol, n-hexane, and water respectively. Filtered with the help of Whatman's no.1 filter paper. For future use stored in glass vials kept at 4ºC before use (Nayak *et al*., 2015).

***In vitro* screening of the botanical extract by poisoned food method:** Antifungal impact against pathogenic fungi was assessed by the poisoned food method (Balamurugan, 2014). Leaves extracts of *C. bonducella* at 5%, 10%, 15%, and 20% concentrations were prepared in water, ethanol, and hexane solvent respectively. Inoculated each Petri plate with a 9 mm diameter disc of *M. phaseolina* from 5 days old culture incubated at 28±2ºC for 7 days. All treatments were in three replicates with untreated control.

The following formula was used for calculating the % inhibition of the mycelial growth over control (Gupta & Tripathi, 2011).

$$\text{Inhibition of mycelial growth (\%)} = \frac{\text{Diameter of control colony} - \text{Diameter of treated colony}}{\text{Diameter of control colony}} \times 100$$

Isolation of *M. phaseolina* from soil: The wet sieving method (with dilution technique) is used to isolate *M. phaseolina* from soil (Shaikh & Ghaffar, 1975).

***In vivo* impact of botanical extract in the control of *M. phaseolina*:** In a pot experiment, 20 ml aqueous extract of *C. bonducella* was drenched in (300g) of soil. Artificially infested seeds of castor with the sclerotia of *M. phaseolina* (10-15 sclerotia/seed) at (0.01 weight/weight) by using gum arabic (2%) as a holding agent, were allowed to grow in that soil. In another set of experiments, non-infested seeds were placed to grow in soil treated with an extract of *C. bonducella*. In control, soil and seeds were untreated.

For 45 days, three replicates of each treatment were used in the experiment, after that plant growth variable viz root length, shoot length, root weight, and shoot weight were assessed. Colonization % was calculated using the formula below:

$$\text{Colonization \%} = \frac{\text{Number of root pieces colonized by a fungus}}{\text{Total number of root pieces}} \times 100$$

Results

In vitro, the extracts of *C. bonducella* in aqueous and organic solvents (ethanol and hexane) significantly reduced the pathogenic fungal population ($p<0.05$). Maximum fungal growth was recorded (62%) in 20% concentration aqueous extract of *C. bonducella*, which was also followed by 20% ethanolic extract of *C. bonducella* (54.09%) and 15% ethanolic extract of *C. bonducella* (50.81%) (Fig. 1). However, the fungal growth was recorded high in the liquid solutions at 5% concentration and in hexane solvent at all concentrations (Fig. 2).

In the pot experiment, *C. bonducella* extracts were used to check the effect on phenotypic characters (root, shoot length, and fresh, dry weight) of the castor plant. It was found that pots treated with the aqueous of *C. bonducella* provide a significant result ($p<0.05$) (Table 1) to promote the growth of castor plants. The mean value of root and shoot length of castor plants was observed in soil treated with an aqueous extract of *C. bonducella* leaves (non-infested seeds). Whereas, root fresh, dry biomass, and shoot fresh, dry biomass were also evaluated. In soil treated with an aqueous extract of *C. bonducella* (infested seeds) fresh biomass of root was recorded high. However, the fresh weight of the shoot was observed high in soil treated with the aqueous extract of *C. bonducella* (non-infested seeds).

While in the case of dry weight high mean values were observed in control plants and soil treated with an aqueous extract of *C. bonducella* (non-infested seeds). Although the highest number of leaves of castor was recorded in control (non-infested and non-treated seeds), however, the elevated colonization was observed in control. Plants grown in soil treated with extract of *C. bonducella* highly reduced the colonization of *M. phaseolina* (Table 1).

Discussion

In the present studies, the food poisoned method was used for the investigation of the antifungal properties of *C. bonducella* against *M. phaseolina*. The results showed that the antifungal activity of *C. bonducella* caused a reduction in the mycelial extension of the fungi in the poisoned plate when compared to the control plate. It has been reported the various result to assess the antifungal activity of plants (Ortiz *et al*., 2010). According to the results of the study, the extract of *C. bonducella* at a concentration of 20% was found to be more effective in suppressing the growth of *M. phaseolina* compared to the ethanol extract at concentrations of 20% and 15%. This suggests that the higher concentration of the *C. bonducella* extract may have stronger antifungal properties against *M. phaseolina*. The observed antifungal activity of *C. bonducella* extracts against *M. phaseolina* can be attributed to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds (Sasidharan *et al.*, 2022). These bioactive constituents possess inherent antifungal properties and can disrupt the growth and development of fungal pathogens. The ethanol extract, being the most effective, might contain a higher concentration of these phytochemicals (Adegboye *et al.*, 2021; Sukumar *et al.*, 2020).

The results of the current research indicate that the growth parameters of castor plants, such as shoot biomass and root biomass, were significantly improved. In the study, the soil was treated with an aqueous extract of *C. bonducella*, and the seeds were artificially infested with *M. phaseolina*. The results showed that the colonization percentage due to the pathogenic fungi was reduced. The mechanism of antifungal action of *C. bonducella* extracts could involve multiple modes, including inhibition of fungal cell wall synthesis, disruption of membrane integrity, interference with vital enzymatic activities, and induction of oxidative stress leading to reactive oxygen species production (Sukumar *et al.*, 2020; Kumar *et al.*, 2019). Extracts of *C. mas*, *M. nigra* and *P. laurocerasus* were reported to prevent the growth of mycelium of walnut anthracnose between 46.98 and 67.95% (Kalkisim, 2012). *Rosa canina* exhibits antioxidant and antibacterial effects against Gram (+) and Gram (-) bacteria and antifungal activity against *Candida* spp. (Orhan *et al.*, 2012; Martinsa *et al.*, 2015).

Table 1. Impact of *C. bonducella* on plant growth parameters and colonization % of *M. phaseolina.*

Treatment	No. of leaves	Root length (cm)	Shoot length (cm)	Root biomass (g)		Shoot biomass (g)		Colonization %
				Fresh	Dry	Fresh	Dry	
Control	06 ± 00	6.87 ± 0.94	14.50 ± 3.01	1.63 ± 0.14	1.07 ± 0.11	3.90 ± 0.31	1.29 ± 0.05	4.44 ± 2.22
C. bonducella	04 ± 00	11.33 ± 2.68	11.83 ± 0.44	1.22 ± 0.07	0.05 ± 0.03	3.38 ± 0.45	0.30 ± 0.20	0.00+0 ± 0.00
C. bonducella + *M. phaseolina*	02 ± 00	6.23 ± 0.83	10.63 ± 0.32	2.05 ± 0.34	0.33 ± 0.14	3.28 ± 0.26	0.33 ± 0.06	2.22 ± 2.22
F-value	5.44*	1.25^{ns}	2.65^{ns}	3.72^{ns}	25.00**	0.90^{ns}	19.78*	3^{ns}

Note ± Standard error. Sig. (P) = Significance level: *p<0.05, **p<0.01 and ***p<0.001 ns = Non-significant

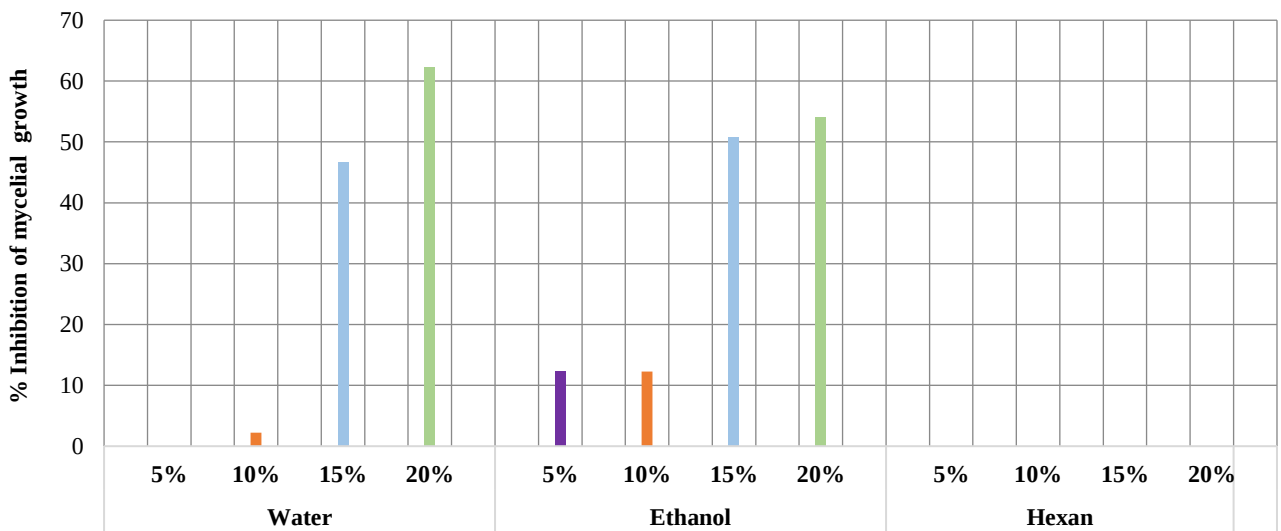

Fig. 1. % Inhibition of mycelial growth of *M. phaseolina* by *C. bonducella* at different solvents and concentrations.

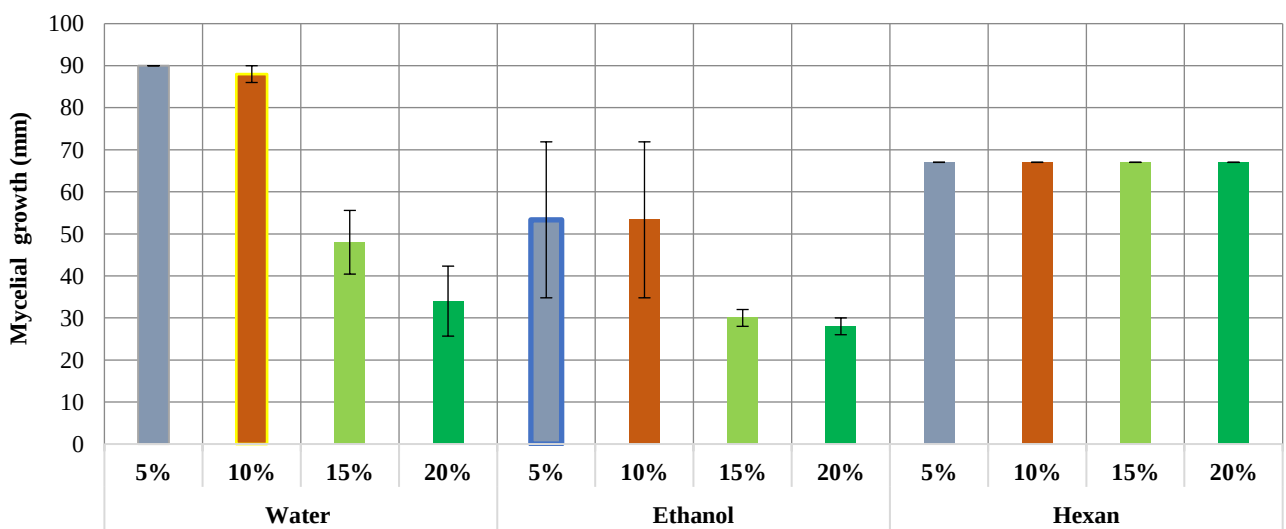

Fig. 2. Mycelial growth of *M. phaseolina* at different solvents and concentrations of *C. bonducella*.

Conclusion

The study has shown that the medicinal plant *C. bonducella* is very efficient and ideal for preventing the mycelial growth of *M. phaseolina*. This plant could also be utilized in field trails to determine its productivity in field trail as well. It can be concluded from all results of the present finding that phytopathogenic fungi *M. phaseolina* both *In vitr*o and *In vivo* conditions tested against leaves extract of *C. bonducella*. It was discovered that plant extract had the most impact on the activity of *M. phaseolina* and mycelial growth was severely inhibited.

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