

LABORATORY EVALUATION OF FUNGICIDES AND PLANT EXTRACTS AGAINST DIFFERENT STRAINS OF *Colletotrichum falcatum* THE CAUSE OF RED ROT OF SUGARCANE

Hafiz Tassawar Abbas^{1,*}, Shahbaz Talib Sahi¹, Amer Habib¹ and Sohail Ahmed²

¹Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan;

²Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan.

*Corresponding author's e-mail: khantassawar@gmail.com

Red rot incited by *Colletotrichum falcatum* (Went.) is the most destructive disease of sugarcane in Punjab and Sindh provinces of Pakistan. Laboratory studies were conducted using CRD with three replications to study the efficacy of six fungicides viz. Captan, Carbendazim, Copper Oxichloride, Mancozeb, Tilt, Topsin-M and plant extracts *Allium sativum*, *Azadirachta indica*, *Datura stramonium*, *Eucalyptus camaldulensis*, *Moringa oleifera* and *Zingiber officinale* against red rot of sugarcane. Maximum inhibition (95%) of the strain-234 was expressed by Mancozeb at 3% concentration followed by Tilt, Carbendazim, Topsin-M and Copper Oxichloride and minimum inhibition was observed by Captan (37.3%) at 1% concentration as compared to other strains. Least inhibition (32.2%) of strain-234 was exhibited by the extract of *Eucalyptus camaldulensis* at S/25 concentration and maximum growth was suppressed by *Azadirachta indica* (89.9%) at S (Standard dose) concentration followed by *Datura stramonium*, *Moringa oleifera*, *Allium sativum* and *Zingiber officinale* respectively. The present study suggested that growth of the pathogen is effected by different concentrations of fungicides and plant extracts may play an important role to manage this disease.

Keywords: Sugarcane, strains, *Colletotrichum falcatum*, fungicides, plant extracts, red rot.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L) is an economically important cash crop of Pakistan (GOP, 2014) which is grown in tropical and sub-tropical areas of world (Chatenet *et al.*, 2001). Cane syrup, ethanol, rum and wax are the main by products derived from sugarcane whereas molasses are used as sweetener, alcohol fermentation and feed stalk. (Mackintosh 2000; Sansoucy *et al.*, 1988). It covers an area of over 1.17 million ha with an average productivity of 66.5 million tons ha⁻¹ (GOP, 2014) which is quite low when compared with other major sugarcane producing countries of the world like Brazil, India, China, Thailand and Mexico. Pakistan ranks 4th in sugarcane acreage and conquers 5th position in sugar production worldwide (Shahina *et al.*, 2007).

More than 100 diverse diseases have been reported in sugarcane which are caused by fungi, bacteria, viruses, nematodes and phytoplasma (Bharti *et al.*, 2012). A number of sugarcane diseases like whip smut, pokkah boeng, red rot, sugarcane mosaic virus, red and yellow stripes and rust are reported in Pakistan (Anwar *et al.*, 2010). Among these, red rot of sugarcane is most important one caused by *Colletotrichum falcatum*. It is demonstrated by numerous references to its economic importance as the world has witnessed severe red rot epiphytotic at different times. It was first reported by Went in Java (Indonesia) in 1893 (Went, 1893) which was later on

renamed as red rot by Butler in 1906. In Pakistan, it was first described in 1986 (Ahmed *et al.*, 1986). It is responsible for 5-10% yield, 30 to 87% juice, 28.5 to 82.7% cane weight reduction and 30 to 74% in expected sugar recovery (Ahmed *et al.*, 1986). *Colletotrichum falcatum* hydrolyses the stored sucrose by producing the enzyme invertase which breaks the sucrose molecule into glucose and fructose resulting into increased molasses (Sehtiya *et al.*, 1993). The ideal way to combat the disease is the development of the resistant sugarcane lines/varieties (Gupta *et al.*, 1982; Viswanathan *et al.*, 1996; Malathi *et al.*, 2008). Fungicides play a vital role in disease management because they control many diseases satisfactorily (McGrath, 2004). Cowan (1999) recorded that Plants contain extensive variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, having antimicrobial properties. Growth inhibition of *C. falcatum* was observed by various plant extracts (Imtiaj *et al.*, 2007). The efficacy of plant extracts of *Azadirachta indica* and *Allium sativum* against *Colletotrichum spp* has been expressed by Kumar and Yadav (2007). Keeping this in view, recent study was accomplished to find out the role of best fungicide and antifungal plant extract which have inhibitory effect against different strains of *C. falcatum* of red rot disease of sugarcane.

MATERIALS AND METHODS

Evaluation of fungicides and plant extracts were done with different concentrations of fungicides and plant extracts against strains of *C. falcatum* following standard poisoned food technique.

Impact of fungicides against strains of *C. falcatum*: Sensitivity of four strains *C. falcatum* to six fungicides Captan, Carbendazim, Copper oxichloride, Mancozeb, Tilt and Topsin-M were tested by following 'Poisoned Food Technique' (Nene and Thapliyal, 1982). Fungicides description was given (Table 1). Requisite quantity of active ingredient of each fungicide was mixed in autoclaved oat meal agar separately to obtain the required concentrations of 1, 2 and 3 % respectively. Poisoned medium (20 mL) was poured into 9 cm Petri plates. After solidification of medium, plates were inoculated with five mm discs obtained from the periphery of a seven day old culture for each of the four strains of *C. falcatum* separately. Petri plates were incubated at $30 \pm 2^\circ\text{C}$ for 10 days. Unamended medium served as control. The experiment was conducted in completely randomized design (CRD) with three replications and observations for each of the three levels were recorded. The results were expressed as per cent inhibition of the mycelial growth over the control by using formula given by Bhardwaj and Sahu (2014) after 2, 4, 6, 8 and 10 days respectively for each strain of *C. falcatum*.

$$\text{Percent inhibition} = \frac{X-Y}{X} \times 100$$

Where, X = Colony diameter in check, Y = Colony diameter on amended medium

In vitro evaluation of plant extracts against strains of *C. falcatum*: Six plants extract *Allium sativum*, *Azadirachta indica*, *Datura stramonium*, *Eucalyptus camaldulensis*, *Moringa oleifera* and *Zingiber officinale* were tested for their efficiency to reduce the mycelia growth of four strains *C.*

falcatum. Plants description was given in Table 2. For antifungal mechanism of plant extracts, the poisoned food technique was used. Fresh leaves (75 gram) of each tested plant were macerated in 75 mL of distilled water using sterilized pestle and mortar. The macerated leaf extract were passed through Whatman filter paper. This prepared dose was considered as (S) standard dose (Ilyas *et al*, 1997). Similarly S/50 concentration was made by mixing half part of standard dose with 100 mL of sterilized water and to make S/25 concentration, half part of S/50 with 100 mL of sterilized water was used separately. The plant extracts were thoroughly mixed with sterilized molten oat meal agar (OMA) medium. The medium was thoroughly shaken for uniform mixing of each extract with chloramphenicol to avoid bacterial growth. Amended medium (20 mL) was poured into sterile Petri plates. After solidification of medium, plates were inoculated with five mm discs obtained from the growing edges of seven day old culture for each of the four strains of *C. falcatum* separately. This disc was placed in the well on the center of each Petri plate containing amended OMA medium which was made by the help of sterile cork borer. The medium with Inoculum disc but without any plant extract served as control. Then such Petri plates were incubated $30 \pm 2^\circ\text{C}$ for 10 days. The experiment was conducted in completely randomized design (CRD). Each treatment was replicated three times. The percent inhibition of mycelial growth was determined by the following formula given by Bhardwaj and Sahu (2014) after 2, 4, 6, 8 and 10 days respectively for each strain of *C. falcatum*.

$$\text{Percent inhibition} = \frac{X-Y}{X} \times 100$$

Where, X = Colony diameter in check, Y = Colony diameter on amended medium

The recorded data was interoperated by statistical analysis. All statistical tests were performed by using MINITAB

Table 1. Fungicides description.

Sr. No	Fungicide	Chemical name	Formulation	Manufacturer
1	Captan	Captan	50% WP	ICI (Pvt) Ltd.
2	Derosal	Carbendazim	50% WP	Bayer (Pvt) Ltd.
3	Cobox	Copper oxichloride	50% WP	Pak Agro
4	Dithane M-45	Mancozeb	80% WP	Dow Agro Sciences
5	Tilt	Propiconazole	250% EC	Syngenta
6	Topsin-M	Thiophanate-methyl	70% WP	UPI

Table 2. Plants description.

Sr. No	Common name	Botanical name	Family	Plant part used
1	Garlic	<i>Allium sativum</i>	<i>Amaryllidaceae</i>	Leaves
2	Neem	<i>Azadirachta indica</i>	<i>Meliaceae</i>	Leaves
3	Datura	<i>Datura stramonium</i>	<i>Solanaceae</i>	Leaves
4	Eucalyptus	<i>Eucalyptus camaldulensis</i>	<i>Myrtaceae</i>	Leaves
5	Sohanjana	<i>Moringa oleifera</i>	<i>Moringaceae</i>	Leaves
6	Ginger	<i>Zingiber officinale</i>	<i>Zingiberaceae</i>	Leaves

statistical software (Minitab, 2010). Means were separated using Fisher's protected least significant difference (LSD) procedure (Steel *et al.*, 1997).

RESULTS

In vitro evaluation of different fungicides against strains of *C. falcatum*: Significant inhibitory effects of fungicides and their concentrations were observed on the growth of different strains of *C. falcatum*. The minimum inhibition growth of four strains viz. 234, 1148, 394 and 285 was expressed by Captan at 1% concentration (37.31, 32.31, 27.31, 22.31%) after 2 days and maximum inhibition was observed by Mancozeb (95, 90, 85, 80%) at 3% concentration after 10 days followed by Tilt (67.2, 62.53, 57.53, 52.53%), (71.89, 66.89, 61.89, 56.89%), (75.68, 70.68, 65.68, 60.68%), Carbendazim (62.53, 57.53, 52.53, 47.53%), (66.89, 61.89, 56.89, 51.89%), (70.68, 65.68, 60.68, 55.68%), Topsin-M (57.53, 52.53, 47.53, 42.53%), (61.89, 56.89, 51.89, 46.89%), (65.68, 60.68, 55.68, 50.68%) and Copper Oxichloride (52.53, 47.53, 42.53, 37.53%), (56.89, 51.89, 46.89, 41.89%), (60.68, 55.68, 50.68, 45.68%) after 4, 6 and 8 days, respectively. The individual inhibition caused by the fungicides at three concentrations (Fig. 1, 2, 3, 4).

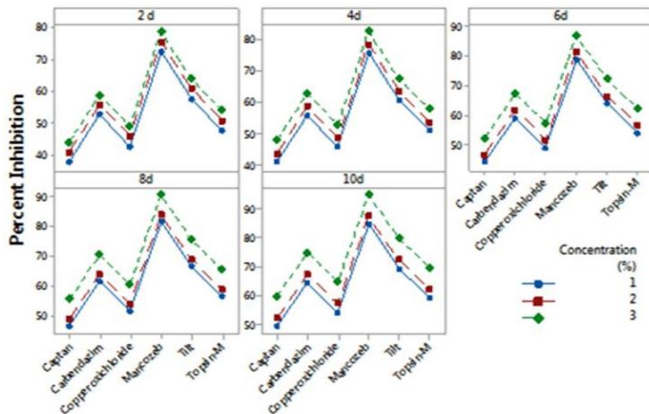


Figure 1. Evaluation of different fungicides against strain-234 after different days interval.

In vitro evaluation of plant extracts against strains of *C. falcatum*: The aqueous extracts of all the tested plants significantly suppressed the growth of different strains of *C. falcatum*. *Azadirachta indica* (89.90, 79.90, 74.90, 69.90%) found to be the most effective in suppressing the growth of the four strains 234, 1148, 394 and 285 after 10 days followed by *Datura stramonium* (62.43, 57.43, 52.43, 47.43%), (66.79, 61.79, 56.79, 51.79%), (70.58, 65.58, 60.58, 55.58%), *Moringa oleifera* (57.43, 52.53, 47.43, 42.43%), (61.79, 56.67, 51.79, 46.79%), (65.58, 60.68, 55.58, 50.58%), *Allium sativum* (52.43, 47.43, 42.43, 37.43%), (56.79, 51.79, 46.79,

41.79%), (60.58, 55.58, 50.58, 45.48%) and *Zingiber officinale* (47.43, 42.43, 37.43, 32.43%), (51.79, 46.79, 41.79, 36.17%), (55.58, 50.58, 45.58, 40.58%) at S (Standard dose) concentration after 4, 6 and 8 days and *Eucalyptus camaldulensis* which proved to be least effective at S/25 concentration exhibited the minimum inhibition (32.21, 27.21, 22.21, 17.21%) after 2 days separately. The distinct inhibition caused by the plant extracts at all concentrations (Fig 5, 6, 7, 8). Least inhibition of strain-234 was expressed by *Eucalyptus camaldulensis* (32.21%) at S/25 concentration after 2 days and maximum grow suppressed by *Azadirachta indica* (89.90%) at S (Standard dose) concentration after 10 days followed by *Datura stramonium*, *Moringa oleifera*, *Allium sativum* and *Zingiber officinale* after 4, 6 and 8 days respectively as related to other strains.

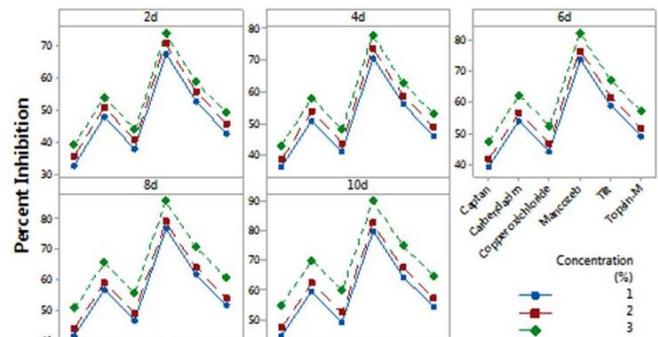


Figure 2. Evaluation of different fungicides against strain-1148 after different days interval.

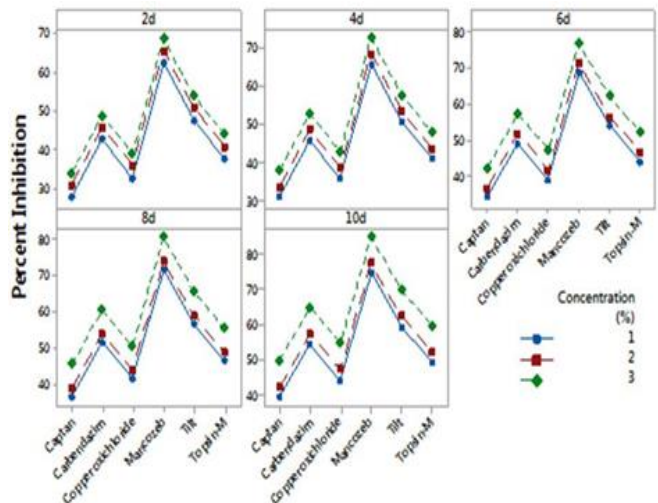


Figure 3. Evaluation of different fungicides against strain-394 after different days interval.

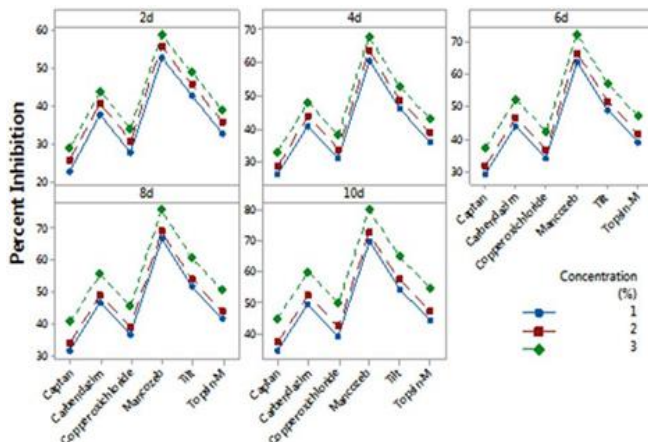


Figure 4. Evaluation of different fungicides against strain-285 after different days interval.

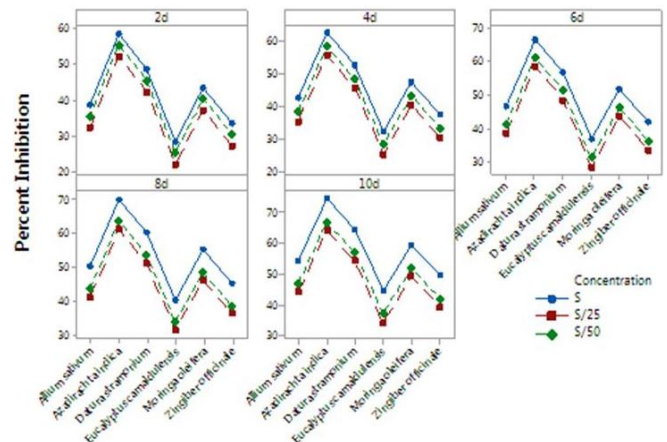


Figure 7. Antifungal potential activity of different plant extracts against strain-394 after different days interval.

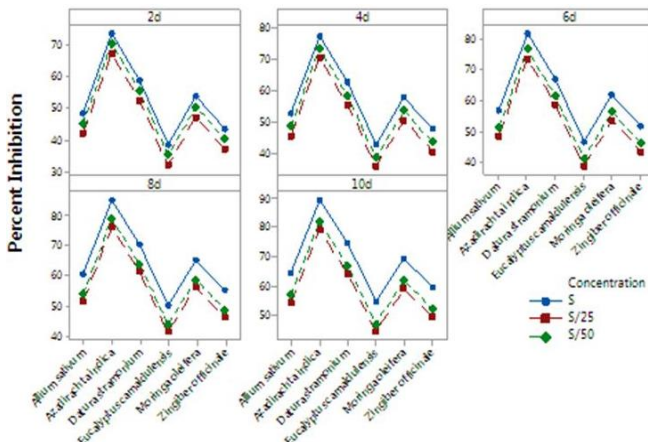


Figure 5. Antifungal potential activity of different plant extracts against strain-234 after different days interval.

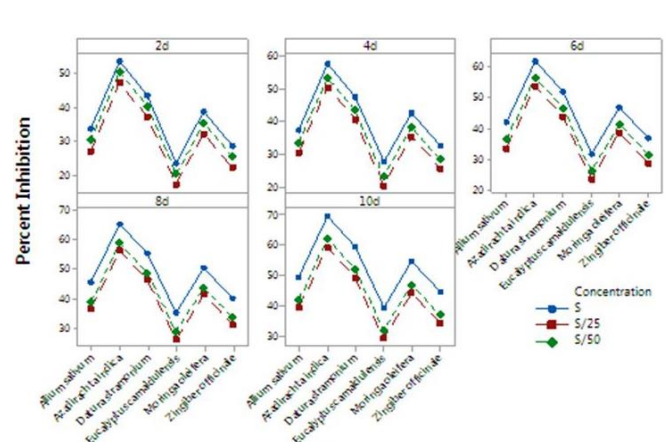


Figure 8. Antifungal potential activity of different plant extracts against strain-285 after different days interval.

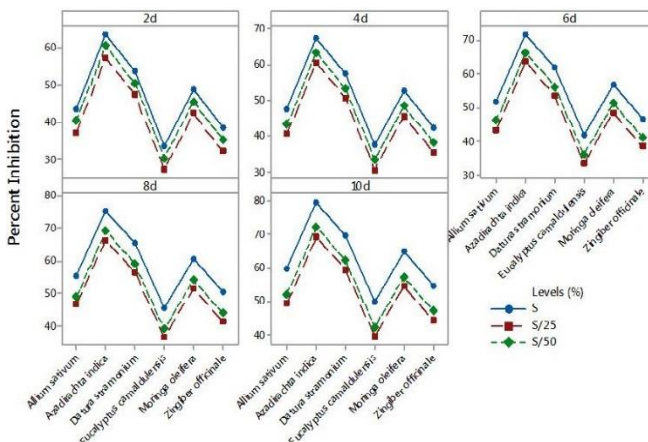


Figure 6. Antifungal potential activity of different plant extracts against strain-1148 after different days interval.

DISCUSSION

Cultivation of resistant varieties is the most economical method to manage red rot of sugarcane (Viswanathan and Samiyappan, 1999; Viswanathan *et al.*, 2009) but when disease free seed is not available farmers are left with no option except sett treatment with some effective chemicals. Chemical control is easy, direct, rapid action and helped to solve disease problems. In the present study *in vitro* evaluation of fungicides against four strains, maximum inhibition of the strain-234 was observed by Mancozeb (95%) at 3% concentration after 10th day incubation as compared to other strains respectively.

The observations of present study are in confirmation with the results published by Imtiaz *et al.* (2007) who reported that Dithane M-45 (Mancozeb) was observed to be the best for percent inhibition colony growth of *C. falcatum* after 10 days.

Hegde (1998) got the similar findings who recorded that Mancozeb (DM-45) was found to be highly effective in inhibiting growth of *Colletotrichum spp.* at a different concentration. Fungicide acts by binding with β -tubulin polymers of pathogens which take part in a key role in nuclear partition and result in reticence of polymerizing activity of microtubules. These also cause barrier in diverse dictatorial cellular activities including mitosis, meiosis and cell form preservation etc. (Nene and Thapliyal, 1982). Mancozeb might be attributed due to this reason exhibited significant relationship.

Our results are partially matched with the results Subhani *et al.* (2008) who determined that red rot of sugarcane (*C. falcatum* Went.) was controlled by twelve fungicides. The (100%) inhibition was also found in case of Tilt 250 EC at different concentrations. Similarly Bhardwaj and Sahu (2014) reported that Carbendazim was found to be most effective against *C. falcatum* followed by Folicur, Contaff and Tilt at different concentrations respectively.

Mesta (1996) has also reported similar results who observed that Mancozeb, was proved to be highly effective inhibiting the growth of *Colletotrichum spp.*

Frequent use of chemicals is neither economical nor beneficial for the environment. Continuous reliance on pesticides has proven unsuitable and in reality has led to grander problems in pest control such as environmental pollution and degradation (Singh *et al.*, 2003). Management of red rot disease of sugarcane through plant extracts and helped to avoid such problems because it is environmentally sound and suitable strategy which seeks to minimize the use of chemicals. In the present experiment different plant extracts were evaluated against *C. falcatum*. Among six plant extracts, maximum growth suppressed by *Azadirachta indica* (89.90%) at S (Standard dose) concentration for strain-234 after 10 days followed by 1148, 394 and 285.

In support of present study, Khan (1989) studied the effect of seed and leaf extracts of neem (*A. indica*) against *Alternaria radicina* and *Helminthosporium turcicum*, *Ascochyta rabiei* and *Macrophomina phaseolina*. Similarly, Dwivedi and Dubey (1986) recorded that the volatile fractions of two medicinal plants like *Azadirachta indica* and *Eucalyptus globulus* showed pronounced effect against *Macrophomina phaseolina*.

Neem leaves contain azadirachtin which possess antifungal properties (Sadri *et al.*, 1983). This might be attributed due to azadirachtin, *A. indica* had showed significant results in the present study. The results are in agreement with Shivpuri *et al.*, (1997) who reported leaf extracts of *A. indica*, *D. stramonium*, *O. sanctum*, *P. longifolia* and *C. roseus* having fungitoxic against *A. brassicola*, *C. capsici*, *F. oxysporum*, *R. solani* and *S. sclerotiorum*.

Mukhtar (2007) reported that the aqueous extracts of four plant species viz; *Datura metel*, *Azadirachta indica*, *Parthenium hysterophorus* and *Ocimum sanctum* were tested

in vitro study. Between these plants extracts, *A. indica* and *D. metel* inhibited the mycelial growth of *F. oxysporum f. sp. ciceri* supporting the present study. Similarly, *A. indica* has revealed that the efficacy against *F. solani*, *C. lunata* and *R. bataticola* on brinjal and sunflower (Hussain *et al.*, 2000; Joseph *et al.*, 2008). Similar findings were expressed by Ahmed *et al.* (2002) who observed that the efficacy of *A. indica* against *Bipolaris oryzae* under *in vitro* conditions.

Conclusion: The study concluded that Mancozeb has a great potential to suppress maximum inhibition against strain-234 at 3% concentration after 10 days and minimum inhibition expressed by at 1% concentration after 2 days as compared to other strains. Similarly *Eucalyptus camaldulensis* expressed least inhibition of strain-234 at S/25 concentration after 2 days and maximum growth was observed by *Azadirachta indica* at S (Standard dose) concentration after 10 days as related to other strains.

Acknowledgment: The research team is highly thankful to Higher Education Commission, Pakistan for financial support as Indigenous Scholarship to the first author.

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