International Journal of Botany
and Research (IJBR)
ISSN(P): 2277-4815; ISSN(E): 2319-4456
Vol. 4, Issue 6, Dec 2014, 1-6

EFFECT AQUEOUS EXTRACT OF *XANTHIUM STRUMARIUM* L AND *TRICHODERMA VIRIDE* AGAINST *RHIZCTONIA SOLANI*

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ABSTRACT

The Present work studied the biological agent *T. viride* (T.V) and antifungal activity of the aqueous extract plant(PE) obtained from rough Cocklebur *X. strumarium* L. against the *R. solani* (R.S) caused by damping-off on Okra in the PDA and greenhouse. That *T. viride* has antagonistic activity against *R. solani* were attain 100% in the PDA. All tested plant extract concentrations had antifungal activity against *R. solani*. Radial growth of *R. solani* decreased significantly with increasing the concentration of plant extract. High growth inhibition was observed at concentration 40% were attain 100% when of the concentration other 0, 10, 20 and 30 % inhibition attain to 0, 62.12, 72.59 and 81.03% respectively. Germination percentage of seeds increasing in treated plants with R.S+T.V+PE treatment which attain 90.00% compared to treated plants with R.S attain 60.00%. Damping-off percentage of seedling reducing in treated plants with R.S+T.V+PE treatment which attain 26.33% compared to treated plants with R.S attain 36.10%. Root and shoot length in treated plants with R.S+T.V+PE treatment which attain 30.92 and 10.54 cm respectively compared to treated plants with R.S attain 26.16 and 7.00 cm respectively.

KEYWORDS: Okra, Aqueous Extract, Antifungal Activity, Biological Agent, *Rhizctonia solani*

INTRODUCTION

Fungal infections cause significant loss in many economic crops. Crop losses are estimated to be about 14% worldwide (Agrios 2005). They cause Rhizoctonia root rot, and stem-rot on a wide variety of agricultural crops, respectively (Yaqub and Shahzad 2005). Chemical control may be available to effectively and extensively reduce the effects of most fungal disease but field application of these chemical fungicides may not al- ways be desirable. Excessive and improper use of these fungicides presents a danger to the health of humans, animals, and the environment. Therefore, an extensive search for biofugicides that are environmentally safe and easily biodegradable have been carried out during the last two decades (Gnanamanickam 2002). The ability of *Trichoderma* spp. to reduce the disease is well known and related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogen and /or competition for limiting factors in the rhizosphere mainly iron and carbon (Chet, 1990). *Trichoderma* species can be very efficient in controlling *R.solani* damping off of various vegetable plants using several mode of actions against the pathogen (Barakat et al, 2007). Weindling (1932) described in detail the mycoparasitism of a fungal pathogen causing damping off disease *Rhizoctonia solani* by the hyphae of *Trichoderma*, including coiling around the hyphae, penetration, and subsequent dissolution of the host cytoplasm. He also described an antibiotic which was toxic to both *R. solani* and Sclerotinia americana, and named it gliotoxin. Integrated pest management (IPM) for conserving agro-ecosystem include the use of pest resistance cultivars, holding pests at tolerable levels and making use of natural products (Rai and Carpinella, 2006).

A number of studies have been conducted to explain the putative traditional medicinal uses of *X. strumarium*. A methanol extract was shown to have in vitro antibacterial and antifungal activities (Jawad et al, 1988). the biological activities of the major bioactive compound, xanthatin, and other compounds from *Xanthium strumarium* (Asteraceae) leaves (Nibret et al. 2011). The investigation of plants containing natural antimicrobial metabolites for plant protection has been identified as a desirable method of disease control (Rai and Carpinella 2006). Various plant products like plant extracts, essential oils, gums, resins etc. were shown toexert biological activity in vitro and in vivo and are used as bio-fungicidal compounds (Fawzi et al. 2009; Al-Askar and Rashad 2010).

MATERIALS AND METHODS

Rhizctonia solani

R. solani was isolated from diseased Okra seedling infected By damping-off and root rot. Were isolated and identified on Potato dextrose agar medium PDA by (Domsch et al. 1980).

Trichoderma viride

T. viride were obtained from the Department of Plant Protection Agriculture College – university of Basra-Iraq. The fungus were culture on potato dextrose agar Medium. the fungus was cultured in the laboratory and preserved. It has been sub-cultured on PDA for 7 days in 28°C and slant were kept in 4°C for further study after 7 days of growth.

Antagonistic Effects of *T.viride* against *R. solani*

Five-millimeter diameter disk obtained from the edge of actively growing colony of *R. solani* were paired on individual plate of PDA with similar disk of *T. viride*. in the control experiment Five-millimeter diameter disk obtained from the edge of actively growing colony of pathogen were placed on center PDA plate the plates incubated at 28°C and measurements were taken after 7 days (Abd-El-Khair et al. 2010) at the end of the incubation period, radial growth was measured radial growth reduction was calculated in relation to growth of the control as follows:

$$\% \text{ reduction} = C - T / C \times 100$$

Where

C=radial growth measurement of the pathogen in control

T= radial growth measurement of the pathogen in the presence of *T.viride*

Collection of Plant Material

The plant Cocklebure *Xanthium strumarium* L. (Asteraceae) was collected in around of Agriculture college. the powdered leafs was used for further studies.

Preparation of Plant Aqueous Extract

The fresh leafs were cleaned, dried and grounded into fine powdered sample 100gm were macerated with 1000ml sterile distilled water in a blender for 10 min. The macerate was first filtered through double-layered muslin cloth followed by centrifugation at 4000rpm for 30 min at room temperature. The supernatant was filter paper and sterilized, which served as the mother extract (Satish et al.2007).

Antifungal Activity of Aqueous Extract

For screening of antifungal activity of powder used poisoned food technique was followed (sinha et al. 1993) potato dextrose agar PDA medium was preparation and sterilized. the medium was supplemented with different serial dilution of aqueous extract i.e. 10, 20, 30 and 40 % from stock solution. about 15 ml of this medium was poured into each petriplate and allowed to solidify five mm disc of 7 days old culture of fungus were placed at the center of the each petriplate and incubation at 28 °C for five days. After incubation the colony diameter was measured in centimeter cm for each treatment group (for a given percentage of extract) three replicates were maintained. PDA medium without the aqueous extract was taken as control. The fungitoxicity of the extract was taken in terms of percentage inhibition of mycelia growth was calculated by using the following formula: % inhibition = dc – dt / dc × 100

Where dc=Average increase in mycelia growth in control

dt = Average increase in mycelia growth in treatment(Singh and Tripathi,1999).

Preparation of Inocula

Three hundred grams(300g) of ground maize were suspended in 500 ml of distilled water containing 15 g of glucose.the suspended was dispensed in 1 liter conical flask and autoclaved at 121C° for 30 mints. The flask were allowed to cool to room temperature before each flask was inoculated separately with seven 5mm disc of young (3to4 day old) cultures of *R.solani* and *T.viride*. The were incubated for five days at 25-28 C°. the content of the flask were aseptically removed using flame sterile metal spatula into clean trays, covered with double –layered clean cheese cloth and sun dried for seven to eight days.dry inocula were stored at room temperature until needed (Okereke and Wokocha ، 2006).

Greenhouse Studies

Fifteen plastic pots (14cm diameter) were filled with heat sterilized field soil at the rate of 1.5kg per pot, the field soil was a sandy – loam with pH4.8 ; 0.1% total N and 1.6% organic carbon. Inoculation of potted plants with the pathogen was carried out at the following frequencies, same days as treatment, two days before and after the application of plant extract (PE), *T. viride* (T.V) and Okra seeds were sown in treated soil at the rate of 10 per pot. Potted soils were inoculated by carefully scattering seven grams of *R. solani* (R.S) inoculums by hand on the soil surface and covering with sterile soil to a depth of 5 cm. two hundred milliliters of the aqueous plant extracts were application as soil drenches while seven grams of maize-meal inoculums of *T. viride* were used. Pots inoculated without treatment and non-inoculated pots served as control. The potted soil were randomly arranged twice with three replications and disease incidence was determined by recording the percentage of Germination seeds 15 days after sowing as well as percentage of damping- off and root and shoot length 45days after sowing.

STATISTICAL ANALYSIS

Experiments with three replicates in three plants were evaluated for each replicate. The results were calculated taking control as 100% to find increase or decrease of various activities. The data were analyzed by one-way analysis of variance (ANOVA). The treatment means were compared by F-values, with level of significance $P<0.005$.

RESULTS

Antagonistic Effects of *T. viride* against *R. solani*

The antagonism of *T. viride* against *R. solani* was tested by the dual culture method. It is evident from the results

Figure 1,

Figure 1: Antagonistic Activity of *Trichoderma viride* against *Rhizctonia solani*

Antifungal Activity of Aqueous Extract

The growth reduction 0f *R. solani* in response to the tested plant extract concentrations presented in Figure 2. All tested plant extract concentrations had antifungal activity against *R. solani*. Radial growth of *R. solani* decreased significantly with increasing the concentration of plant extract. High growth inhibition was observed at concentration 40% were attain 100% when of the concentration other 0, 10, 20 and 30 % inhibition attain to 0, 62.12, 72.59 and 81.03% respectively. the concentration 40% was selected to the application in pot experiment under greenhouse conditions.

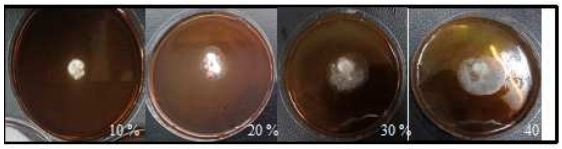

Figure 2: *Invitro* Antifungal Activity of the Plant Extract

Greenhouse Studies

Germination percentage of seeds, Damping-off percentage of seedling and Root and Shoot length were calculated and tabulated in Table 1. Germination percentage of seeds increasing in treated plants with R.S+T.V+PE treatment which attain 90.00% compared to treated plants with R.S attain 60.00%. Damping-off percentage of seedling reducing in treated plants with R.S+T.V+PE treatment which attain 26.33% compared to treated plants with R.S attain 36.10%. Root and shoot length in treated plants with R.S+T.V+PE treatment which attain 30.92 and 10.54 cm respectively compared to treated plants with R.S attain 26.16 and 7.00 cm respectively. Effects of *Trichoderma* spp. against disease has been

Table 1: Effect of Plant Extract and *T. viride* in Suppressing of Pathogenicity of *R. solani* on Okra Seedling under Greenhouse Conditions

Treatments	Percentage %		Length Cm	
	Germination Seeds	Damping-Off	Shoot Length	Root Length
R.S	60.00	36.10	26.16	7.00
R.S +T.V	73.30	28.86	28.20	9.16
R.S +PE	80.13	29.13	27.11	8.28
R.S+T.V+PE	90.00	26.33	30.92	10.54
Control	93.30	6.66	32.43	11.00
R.L.S.$D_{0.05}$	5.37	5.08	0.73	1.29

DISCUSSIONS

That *T. viride* has antagonistic activity against *R. solani* were attain 100%. Antagonism of *Trichoderma* species against *R.solani* has been reported by several workers.(Elad,1996 ; El-katatny et al,2001 ; Howell, 2002 ; Mahamune and Kakde, 2011) Stated that the mechanisms of the antagonism of *Trichoderma* spp. against different pathogen may be due to mycoparasitism, Competitation and antibiosis. The leaves aqueous extract showed potent antifungal activity against

mycelia radial growth of *R.solani* on PDA. The results was therefore in agreement with previous study showing the antifungal against many fungi such as aqueous extract of *X. strumarium* anti- *phytophthore drechsleri* activity as stated by (Bahraminjad et al. 2012). reported by several workers.*Trichoderma viride* reduced of emergence damping-off caused by *R.solani* on Bean plant (Nashwa et al.2008). Trichoderma spp. Significantly increased bath size and weight of Bean plant under greenhouse conditions (Elad et al. 1980). Plant extract as potential antifungal substance has been explored against several fungal diseases. *X. strumarium* contain in their structure a series of compound such as glycosides and phytosterols which have antifungal activity (Kamboj and Kumar ، 2010). Aqueous extract of *Xanthium* spp. Were found to inhibit mycelia growth of *Aspergillus fluvas* (Muhmmoud, 1999).

CONCLUSIONS

Outcomes of our study indicate that *T. viride* have the potential to inhibition the *R. solani* in the PDA media. and antifungal activity of the aqueous extract plant(PE) obtained from rough Cocklebur *X. strumarium* L. against the *R. solani* (R.S) caused by damping-off on Okra in the PDA. The tested both of the *T. viride* and aqueous extract of Cocklebur are potent inhibition further greenhouse studies against *R. solani* causing damping off disease**.**

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