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Efficacy of Clove extract against *Rhizoctonia solani* Causal of Black Scurf of Potato

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ABSTRACT

Potato is one of the most vegetable crops consumed in the world. Many cultivars of potato infested by Black scurf disease (*Rhizoctonia solani*) and affect the quantity and quality of the yield. An experiment was conducted to study the effect of clove extract for controlling *R. solani*. Six isolates were obtained from infected potato plants from different potato growing areas. The results obtained from the pathogenicity test cleared that lady rosetta potato cultivar was the most susceptible cultivar to *R. solani* as affect number of plants. While the most resistant cultivar to *R. solani* during the Pathogenicity test was cara potato cultivar as affected by *R. solani* as affect the mean number of plants. On the other hand, among all six *R. solani* isolates tested during the pathogenicity study Rs4 was the most pathogenic isolate on potato plants while the lowest pathogenic isolate was Rs6 on the tested potato cultivars as affecting the number of plants/ pots. The results for the effect of clove extract (alcoholic and water extract) showed that alcoholic extract was very effective to inhibit the growth of all six *R. solani* isolates as the pathogen was totally inhibited while clove water extract was less effective for inhibiting the growth of the pathogen comparing with control treatment.

INTRODUCTION

Potato (*Solanum tubersum* L.) is the most consumed crop in the world, with an estimated 374 million tons of production worldwide, with a cultivated area of about 17,623,660 hectares(Faostat, 2018). While Potato is ranked one of the most vegetable crops in Egypt, with a total production of 4,325,478 tons from around 163,939 hectares, making Egypt the second largest potato producer in Africa. Potato plants infested by many pathogens affect roots, stolen, stems and tubers.

Rhizoctonia solani is one of the most important fungal pathogen causing a great reduction in both quality and quantity of potato yield (Escande and Echandi, 1991&Jeger *et al*., 1996). It is responsible for delaying the emergence of stems, lesions on stems (stem-canker), and sclerotial formation on potato tubers (black scurf) (Anderson, 1982) the attacking stolons may induce the development of miss-shaped tubers (Escande and Echandi, 1991 & Jeger *et al.*, 1996).

The disease has two phases, viz. stem canker and black scurf. The stem canker phase is the girdling on the stem with brown color and sometimes upward rolling of the leaves also observed. The black scurf symptom is the formation of sclerotia on the surface of the tubers. This phase is more common in the field, particularly at the plant senescent stage. *Rhizoctonia solani* has a wide host range and it is soil and seed-borne in nature. Seed treatment by chemicals is effective against seed-borne scurf **(**Lal *et al*., 2014**).**

Read and Hide, 1995found that seed dressing with fenpiclonil and propiconazole reduced the black scurf symptoms on roots, stem bases, and tubers early in the crop season. The disease is also managed through seed treatment with benomyl, carbendazim, thiabendazole, pencycuronetc (Banyal, 2002) and (Thind and Aggarwal, 2008). Fungal disease control through the use of fungicides is hazardous and toxic to both people and animals and leads to environmental pollution(Abdel-Kader *et al.*, 2012). The extract of weedy plants namely *Lantana camara* and *Capparis decidua* have been used to manage *R. solani* (Sharma and Sarjeet, 2009). Due to safe and non-phytotoxicity may higher plant natural products have been successfully used in disease control.

The use of plant extracts for the management of black scurf disease of potato is a good alternative to chemicals due to their less negative impact on the environment (Sneh and Adams, 1996). Farzaneh *et al.*, 2006tested Artemisia oil against *R. solani* and reported that the oil was exhibited high antifungal activity.(Hassanein *et al*., 2008) tested leaf extracts of neem (*Azadirachta indica*) and chinaberry (*Melia azedarach*) extracted by ethanol, ethyl acetate and water against two tomato fungal pathogens and found that both ethanol and ethyl acetate extracts of neem leaves assayed at a concentration of 20%, completely suppressed the growth of *F. oxysporum* and inhibited *A. solani* by ratios between 52.44 and 62.77% respectively. (Shirzadian *et al*., 2009)evaluated extracts of 23 plants obtained by ethanol, water, and petroleum ether solvents against 7 pathogenic fungal pathogens and found that ethanolic extracts of 6 moss species (*Philonotism archica*, *Grimmia pulvinata*, *Plagiomnium rugicum*, *Haplocladium* sp., *Bryumpallens* and *Drepanocladus aduncus*) followed by two liverworts (*Pelliaepiphylla* and *Dumortiera hirsute*) had more antifungal activity than their aqueous extracts. The current study aimed to isolate the causal pathogens of the black scurf of potato, the effect of plant extracts on *R. solani* pathogen *in vitro* and *in vivo.*

MATERIALS AND METHODS

The present experiments were carried out at the Agricultural Botany and Plant Protection Departments, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt.

1.Isolation of *R. solani*:

Six isolates were obtained from potato tubers with black scarf symptoms from the different locations in Egypt. The infected samples were obtained from Wady EL-Natron (Spounta cv.) (Rs1), Dakhlyia farm (Spounta cv.) (Rs2), Chipsy company (Caruso1 cv.) (Rs3), Dakhlyia farm (Arezona cv.) (Rs4)., Chipsy company (Rosetta cv.) (Rs5), and Chipsy company (Caruso cv.) (Rs6). The collected samples were carried to the laboratory for isolation of the pathogen on the Potato Dextrose Agar (PDA) medium. Isolation and purification have been done for six isolates of *R. solani* which are used for further studies.

2.Pathogenicity Test:

Pathogenicity tests were carried out under greenhouse conditions. The fungal inocula were prepared in 500 ml conical flasks containing cornmeal–sand medium. Each flask contained clean sand (15 g), barley (150g), and enough tap water to cover the prepared mixture and autoclaved for 45 minutes at 121.5 ºC. The flasks were inoculated with each of the isolated fungi and incubated at 27ºC (Whitehead, 1957)

All the *R. solani* isolates were grown on prepared media for 5 days until fungal growth was observed in the media. After five days, 5 g of each isolate was inoculated in one kg of soil in plastic pots (15cm in diameter) and covered by nylon sheet for 4 days, then sowing the infected soil by potato. The disease incidence on potato was recorded according to (Bheemaraya, 2014)after 15 weeks from planting**.**

3. Effect of Plant Extract on *R. solani*:

3.1. Preparation of Plant Extracts:

Clove (*Syzygium aromaticum*)was used in this study and extracted according to the method described by (Al-Manhel and Niamah, 2015)

3.2. Evaluating the Plant Extracts as Antifungal *in vitro*:

1. One ml of plant extract was incorporated into PDA medium just before pouring in sterilized petri dishes. The control treatment was only media without extract.
2. Five concentrations were obtained from the plant extract as (20, 40, 60, 80 and 100%).
3. Add 10 ml of PDA to petri dishes with shaking for mixing.
4. Five mm. diameter discs of *R. solani* were added at the middle of petri dishes, using three replicates.
5. All Petri dishes were incubated for 4 days at 27 ^{o}C.

After 4 days, the radial growth of *R. solani* was measured and the inhibition percentage of the pathogen was calculated by the formula:

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

Where,

I = Percent inhibition in growth of the tested pathogen.

C = Radial growth of pathogen (cm) in control.

T = Radial growth of pathogen (cm) in treated plates

4. Statistical analysis

Data were statistically analyzed as a Factorial in Randomized Complete Block Design(RCBD) design with three replicates according to (statistical analysis system) (SAS) to (Gomez and Gomez, 1984) Least significant differences values (LSD) at 0.05 level of probability was used to compare the differences between treatment means.

RESULTS AND DISCUSSION

1.Pathogenicity of *R. solani* on Potato Cultivars:

In this study, six isolates of *R. solani* were tested for pathogenicity on three potato cultivars (Spunta, Cara and Lady Rosetta) were used throughout the present study. The data presented in Figure 1 showed that there were significant differences among the tested cultivars (Spunta, Cara, and Lady rosetta) as affected by *R. solani* isolates. The number of plants was detected after the treatment by different isolates of *R. solani* on potatoes cultivars. In case of Spunta cv. The results were showed that the highest pathogenicity was recorded by *R. solani* (Rs4) on the mean number of plants (1.00), whereas, the least effect was achieved by (Rs2), (Rs3) (Rs5), and (Rs6) with the nearly the same effect on a number of appeared plants (1.67). For the potato cultivar Cara, the highest pathogenicity was recorded by (Rs4) on a number of plants of potato (0.0), while the least effect was recorded from (Rs1) (2.67). on the other hand, the results for the third potato cultivar (Lady Rosetta) showed that the highest pathogenicity was achieved by Rs4 and Rs5 on a number of the plants (0.67) for both isolates. While the lowest pathogenicity isolates were recorded by Rs6(2.33). According to these results presented in (Fig. 1), a significant reduction was detected in the plant number which was induced by Rs4 (0.56), followed by Rs3 (1.44), Rs2 and Rs5 (1.56),

Rs1(2) and Rs6 (2.11) respectively. And also, there was a significant response between the tested cultivars (Spunta, Cara, Lad yrosetta).

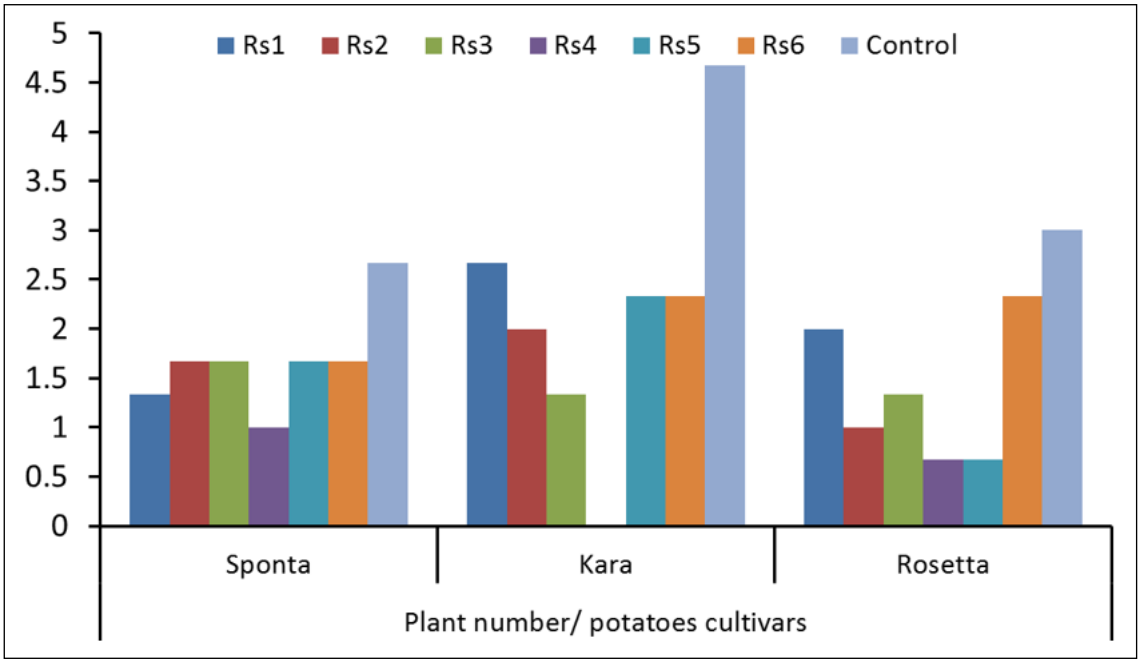

Fig. 1. The efficiency of tested *R. solani* on disease incidence on potato cultivars as a number of plants**.**

Data presented in Figure 2 showed the effect of different isolates of *R. solani* on potato plant height. The results were obtained cleared that the highest pathogenicity was recorded by *R. solani* (Rs3) against the mean number of the plant of potato cultivar (Spounta) (7.00), whereas, the least effect was achieved by (Rs1) (14.33). For the potato cultivar Cara, the highest pathogenicity was recorded by (Rs4) against the mean number of plant height of potato (0.0), while the least effect accrued by (Rs1) (15). finally, when studied the efficiency of different isolates of *R. solani* against potato cultivar (Lady Rosetta). The highest pathogenicity was achieved by Rs4 against the mean number of plant height (3.67). But the least effect was recorded by Rs5 against the mean number of plant height (11). Many investigators such as Vimla *et al*. (2018) found that all the tested isolates caused symptoms of banded leaf and sheath blight (BLSB) on maize and were also cross infective on rice and sugarcane hosts, but showed significant variability pathogenicity and expression of symptoms.

According to these results presented in (Fig 2), a significant reduction was detected in the plant height which was induced by Rs4 (4.36cm), followed by Rs3 (8.67 cm), Rs5 (10.89 cm), Rs2(11.22 cm), Rs6 (11.78 cm), Rs1 (12.78cm) respectively.

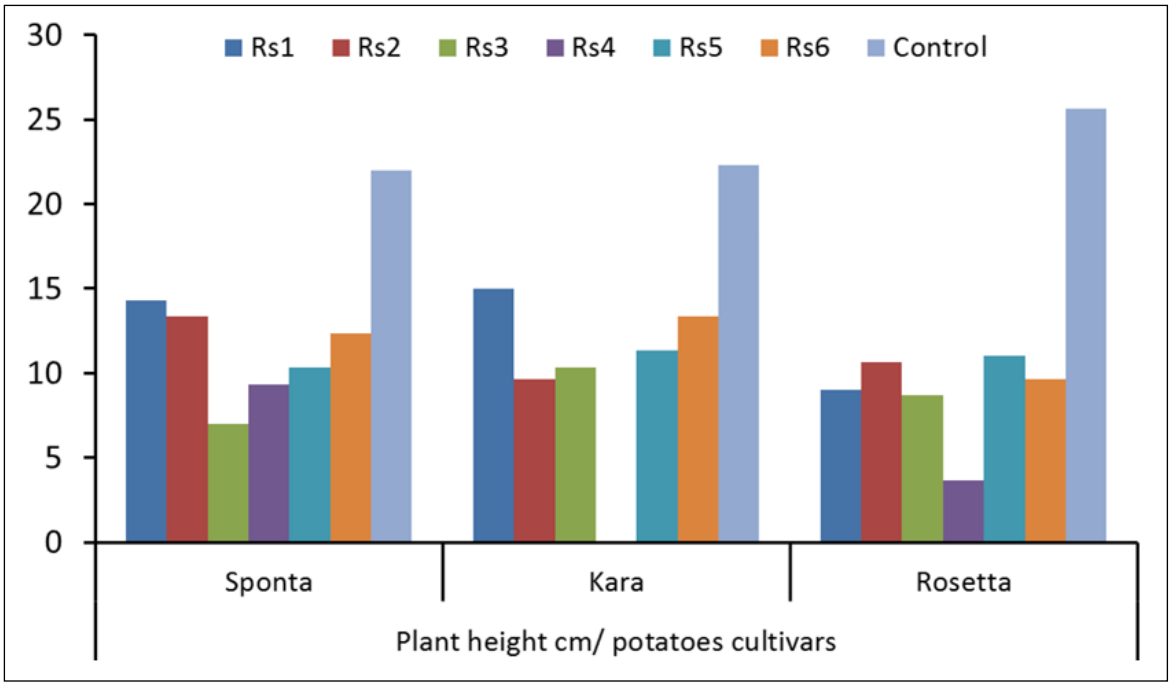

Fig. 2. The efficiency on plant height of potato cultivars infesting by *Rhizoctonia solani*

2.Effect of clove extract with different concentrations on *R. solani* isolates

Data showed in figure 3 cleared the effect of clove extract on the growth and development of six isolates of *R. solani*.

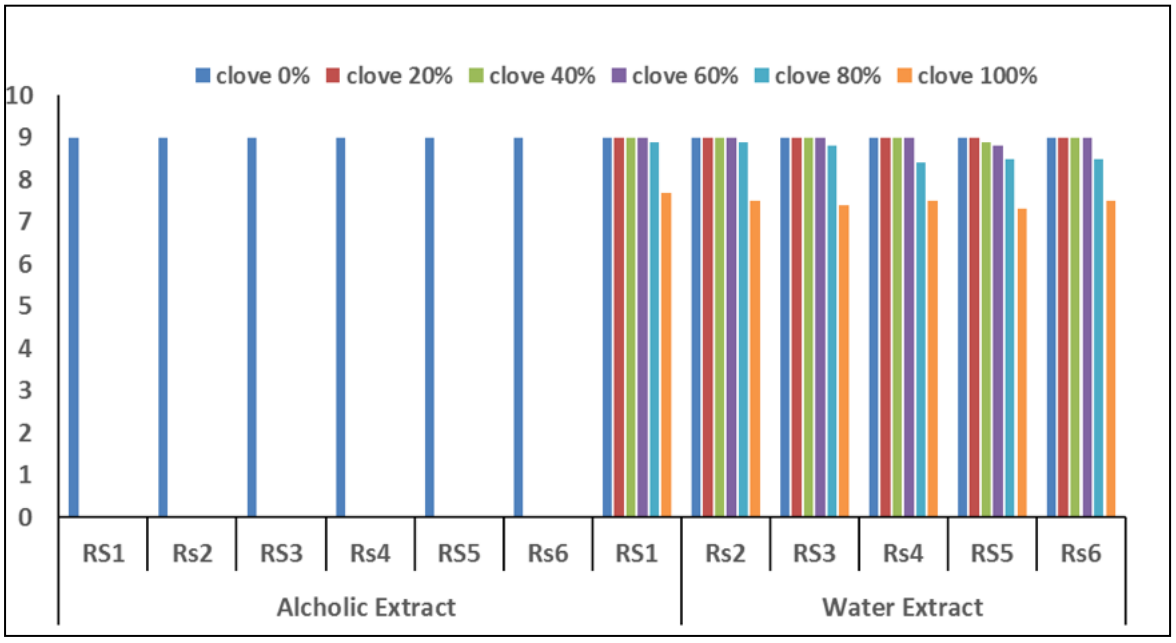

Fig.3: Effect of the different concentrations of clove (alcoholic and water extract) on radial growth(cm) of six isolates of *R. solani.*

The results showed that using different concentrations of alcoholic clove extract (20, 40, 60, 80, 100%) lead to complete suppression for the growth of all *R. solani* isolates comparing to water extract of clove plant which was less effective for inhibiting radial growth of all *R. solani* tested isolates. (Sanaullah *et al.*, 2018)evaluated the effect of the alcoholic extract is better than water extract on *R. solani* isolates on tomato plant. Leaves

extract of three medicinal plants, cinnamon (*Cinnamomum verum)*, moringa (*Moringa oleifera*), and clove (*Syzygium aromaticum*) were investigated against *R. solani* causing damping-off of tomato. Three different concentrations (1%, 2%, and 3%) were used to check the efficacy of plant extracts. Antifungal potency test showed that the maximum growth inhibition was observed at 3% concentration followed by 2 and 1% of each plant extracts. *In vitro* clove extract showed the highest antifungal activity which causes complete mycelial inhibition 3% concentration. At 3% concentration efficacy of plant extracts was also examined on disease incidence as well as plant growth development in greenhouse and field experiments. Clove leave extract at 3% concentration recorded highly significant in disease reduction and other plant growth parameters. Thus, clove leaves are the best choice for managing *R. solani* associated with damping-off disease of tomato. As recorded in this study suggests that essential oils have potential as antimicrobial preservatives for the control of *R. solani* and *Streptomyces* scabies on potato plants in field conditions (Arici and Sanli, 2014).

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