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Management of potato virus Y (PVY) in potato by some biocontrol agents under field conditions

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Abstract

The study was conducted to test the activity of *Pseudomonas fluorescens*, *Rhodotorula* sp and fermented neem extract to protect potato plants against potato virusY disease development under field conditions. Infected potato tubers were soaked in *P. fluorescens*, *Rhodotorula* sp suspensions and in fermented neem extracts separately and sown in the field in completely randomized block design. The development of virus symptoms and the accumulation of virus in the plant based on Enzyme Linked Immunosorbent Assay (ELISA) were followed. The results obtained showed that the treatment of potato tubers with the three agents have significantly accelerated plant emergence, 5-6 days early than non treated ones, and improved plant growth, the plant dry weights ranged from 120-177 g/plant compared to 42 g/plant in non treated plants. The enhancement of plant growth was found associated with reduction in disease severity based on symptoms development and restriction of virus concentration as proved by ELISA absorbance of 405 nm, 0.14-0.23 compared with 2.50 in non treated plants. The results indicated that the use of bioagent to induce systemic resistance provide an efficient tool, as insecticide alternative to manage potato virus Y in potato.

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Introduction

Potato virus Y (PVY), the type member of the genus potyvirus family potyviridae , is among the most important viruses infecting potatoes wherever grown in the world causing heavy losses in the yield (Shukla *et al.,* 1994; Glasi *et al.,* 2002). PVY induce various types of symptoms on potatoes ranging from mild to severe mosaic often associated with leaf necrosis, crinkling, stunting, and leaf drop (Kerlan *et al.,* 1999;Al-Ani *et al.,* 2011). The virus is transmitted by several species of aphids in a non-persistant manner among them *Myzus persicae* was found the most efficient (De Bokx and Huttingo, 1980; Brunk *et al.,* 1996, Kerlan, 2006).

The use of insecticides to manage viruses transmitted by aphids in non-persistent manner was found to be ineffective because the insecticide does not act quickly to prevent virus acquisition or inoculation. In addition the mobility of aphids vectoring the virus during insecticide spray may lead to increase virus dissemination (Satapathy, 1998). Therefore the research was oriented for searching of insecticide alternatives to manage non-persistent transmitted viruses.

It has been reported that plant growth promoting rhizobacteria (PGPR), mainly Pseudomonas species, isolated from rhizosphere of plants promote plant growth by suppressing soil borne pathogens (Bakker *et al.,* 1991;Tuzum and Kloepp,1995; Wei *et al.,* 1996). Some isolates of PGPR found to be able to activate plant defense through inducing systemic resistance (ISR) in plants against broad spectrum of plant pathogens in various plant species (Kloepper *et al.,* 1992; Van Loon *et al.,* 1998). ISR is generally manifested as reduction of disease severity and restriction of pathogen growth compared with non-stimulated control plants (Hammerschmidt, 1999). Treatment of plants with plant extracts can also lead to the induction of resistance to pathogen attack (Walter *et al.,* 2005; Al-Ani *et al.,* 2011).

Recently many species of the yeast Rhodotorula have been reported as effective biocontrol agents against blue and green mold decay leading to reduction of disease incidence and lesion diameter on pear, apple and orange fruit (Quin *et al.,* 2003; Zhang *et al.,* 2007; Zhang *et al.,* 2009, Matny and Faiza, 2012).

As PVY is transmitted by aphids in non-persistent manner and through tubers, and the insecticide sprays often are not effective to manage PVY disease, the objective of this study was to test if bioagents and fermented neem extract that induced resistance against fungal and bacterial pathogens could also protect potato plants against infection by PVY.

Material and methods

Pseudomonas fluorescens isolate

An isolate of *P. fluorescens* was obtained from plant pathology Lab./ Plant protection dept./College of Agriculture /University of Baghdad/Iraq, previously isolated from potato rhizoshere soil . The isolate was grown on nutrient agar (NA) in petri plates at 37 ºC for 24 hrs. A well isolated colony was transferred into 200 ml of nutrient broth in 250 ml Erlenmeyer flasks and maintained at 37 ºC for 48 hrs.

Rhodotorula sp.

Rhodotorula was isolated from local pickle. Hundred µl of pickle was placed on potato dextrose agar (PDA) at 25ºC for 48 hrs. Well isolated colonies were separately suspended in 10 ml of physiological solution (0.85% Nacl) and streaked on PDA using sterile loop. The process was repeated several times for isolates purification. The purified isolates were identified as *Rhodotorula* sp at Food Technologies Department, College of Agriculture, University of Baghdad. An isolate colony was inoculated into 200 ml of nutrient yeast dextrose broth (NYDB) in 250 ml erlenmeyer flask and incubated at 37 C for 48 hrs.

Fermented neem extract

Leaves of neem, approximately 3 kg, were chopped to small pieces of 0.5-1 cm in tight plastic container containing 20 L distill water, 450 ml of effective microorganisms (EM) suspension (purchased from EMRO-CO, Japan), and 450 ml of molasses. The container was maintained under Lab conditions for 10-25 days. The mixture was then passed through muslin cloth and used in the next experiments.

Field experiment

Potato tubers of the susceptible cultivar Desiree infected with potato virus Y (PVY) as proved by Enzyme Linked Immunosorbent assay (ELISA) were collected for the field experiment. The tubers were dipped for 12 hrs in suspensions of *P. fluorescens*, *Rhodotorula* sp at 10^8 CFU/ml and neem extract prepared by addition 200 ml of the extract into 5 L of distilled water (according to company instruction) separately. The tubers were sown in the field in a completely randomized block design with 4 treatments and 3 replicates, 6 plants in each replicate. Infected non-treated and healthy tubers were dipped in distilled water as control. Three month after sowing, three of the youngest leaves of each plant were tested for the presence of viral antigens by ELISA protocol.

ELISA

Potato virus Y was detected in the plants using double antibody sandwich ELISA as described by Clark and Adams (1977). Young upper leaves of plants were homogenized in carbonate buffer (0.03 M NaHCo3, 0.01M Na2Co3, and 0.2% bovin serum albumin (BSA), pH 9.6 (1g:10ml). The homogenate was centrifuged at 5000 rpm for 10 min and 200 ml of the supernatant were loaded in each well of ELISA plate previously coated with anti-PVY IgG at 1.5 µg/ml. The plates were incubated at 37 ºC for 2 hrs and the wells were washed three times with phosphate buffer saline containing 0.05% Tween-20 (PBST). Each well of ELISA plate was loaded with 200 µl of alkaline phosphates conjugated IgG Purchased from BIOREBA AG, Switzerland. Diluted 1:1000 in conjugate buffers (PBST containing 0.2% BSA) and the plates were incubated at 37 ºC for 2 hrs. After washing three time as before, the wells were loaded with 200 µl of substrate (P-nitrophenol phosphate) (PNP) at I mg/ml in 10% diethanlamine, pH 9.8 and the absorbance values were determined at 405 nm within 2 hrs. Absorbance values equal to twice of healthy tissue absorbance values were considered positive.

Results

Results showed that treatment of PVY-infected tubers with *P.fluorescens*, *Rhodotorula* sp and fermented neem extract induced significant reduction in the time of plant emergence associated with significant increase in plant dry weight compared with infected non-treated plants (control). The emergence times and plant dry weights were found to be 15, 16, 15 days, and 120.7, 133.3, 177.0 g/plants for the three agents respectively compared with 21 days at 42.7 g/plant from the control table (1). Symptoms of mild mosaic appeared on the youngest leaves after 2 week of emerging on the control plants, whereas the symptoms on the plants emerged from infected tubers treated with *P.fluorescens*, *Rhodotorula* sp and neem extract were delayed for up to 4 week. The symptom on the treated plants remained mild until the end of the experiment, while those on the untreated plants were developed rapidly to severe mosaic, crinkling and deformation of the new leaves associated with stunting of the plants.

The treatments of infected potato tubers with the bioagents have induced significant restriction in PVY multiplication in the foliage as shown by low absorbance values of ELISA reaction. The absorbance values of ELISA reaction between anti-PVY antibodies and treated from leaves of plants emerged from infected tubers treated with *P.fluorescens*, *Rhodotorula* sp and neem extract were found to be 0.14, 0.23 ,0.18 respectively compared with 2.50 for extract of control plants emerged from infected non-treated tubers (Table 1).

Table 1. Effect of *P.fluorescens*, *Rhodotorula* sp and fermented neem extract on PVY multiplication and plant growth promotion in potato plants.

Treatments	***Absorbance values***	***Germination date/day***	***Dry shoot system/g***
P.fluorescens	0.41	15	170.7
Rhodotorula sp	0.23	16	133.3
neem extract	0.18	15	177.0
Infected non-treated	2.50	21	42.70
Healthy non-treated	0.05	18	141.0
LSD 0.05	1.08	2.85	16.40
SE	0.47	1.12	24.03

* Values in the table represent the mean of 6 reading of ELISA absorbance at 405 nm.

Discussion

The result of this study demonstrated that the treatment of potato tubers with fermented neem extract, *P.fluorescens* and *Rhodotorula* sp have significantly stimulated plant emergence and improved plant growth. Several previous studies reported that fermented plant extracts improve plant growth (Lee and Cho, 1993; Xu *et al.,* 1999; Al-Jarah *et al.,* 2013). The enhancement of plant emergence by the fermented neem extract could results from that, some substances produced during fermentation of neem leaves by the microorganisms may acts as growth promoters, as well as make others more available to uptake by plant roots. Kremer *et al* (2000) reported that effective microorganisms induce decomposition of organic compound to other more easy to be obtained by plant roots.

Similar results were obtained with *P.fluorescens* and *Rhodotorula* sp concerning plant emergence and growth promotion. These results showed similarity with many previous studies where microorganisms have been used to promote germination of many crops (Arsac *et al.,* 1990; Kloepper *et al.,* 1980). The enhancement of plant emergence by *P.fluorescens* and *Rhodotorula* sp may be attributed to the secretion of some substances on the tubers that may activate the biological process and accelerate the emergence. Promoting of plant growth by the bioagents could results from the facilitating uptake of nutrients by roots.

It was reported that PGPR promote plant growth directly through nitrogen fixation, phosphorus solubilization to plant available form and production of phytohormones like auxin ,cytokinin, ethylene, indole-3- acetic acid and gibberellic acid, and indirectly by suppressing soil borne pathogens (Tuzum and Kloepper,1995; Wei *et al.,* 1996; Kim *et al.,* 1998; Vessey, 2003; Pieterse and Van Loon, 2007).

The enhancement of plant emergence and plant growth promotion, triggered by treatment of potato tubers with the bioagents were found associated with reduction in disease severity caused by PVY based on symptoms development and restriction of virus accumulation based on enzyme linked immunosorbent assay (ELISA), compared with non-induced plants infected with PVY. The activity of PGPR against plant pathogens has been reported to be through competition for nutrients, siderophore medicated competition for iron, or antibiosis (Bakker *et al.,* 1991), or indirectly through induce systemic resistance in plants against pathogens (Van Loon *et al.,* 1998). As there is no direct contact between PVY and bioagents used in this study, the resistance manifested in the plant against the virus can be attributed to some form of induced systemic resistance. It was shown that PGPR strain which induced resistance in cucumber against fungal and bacterial disease can also induced resistance in cucumber and tomato plants against cucumber mosaic virus (Racepach *et al.,* 1996).

Conclusions

PGPR and fermented plant extract are very suitable to promote plant growth and mange plant virus disease, because they can be used as seed and seedling treatment or mixed with soil during seedling transplanting. The use of bioagents to induce systemic resistance provides an efficient tool insecticide alternative to mange potato virus Y in potato.

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