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Potato Brown Rot Disease in Egypt: Current Status and Prospects

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Abstract: Potatoes are Egypt's largest horticultural export crop Yet, the total value of Egyptian potato exports fell from a peak value of US$ 102.12 million in 1995 to $US 7.7 million in 2000 mainly due to quarantine restrictions on the potato brown rot imposed by the European Union (EU) which used to account for about 70-90% of Egyptian potato exports. Therefore, The Central Administration for Plant Quarantine (CAPQ) of Ministry of Agriculture, Egypt, has recently set up a new Directorate for Internal Potato Quarantine in order to delimit pest free areas (PFAs), i.e. areas in which *Ralstonia solanacearum* the pathogen of brown rot has not been was known not to have occurred yet . In order for PFAs to be approved, extensive documentation including detailed maps, cropping pattern, irrigation sources and other relevant information must be submitted to the EU Standing Committee on Plant Health. Integrated management of such a disease including cultural, biological and chemical approaches is presented herein. Recognizing that zero tolerance requires sampling every tuber in the potato lot, the default strategy is to define an acceptable tolerance limit. The binomial probability distribution is presented as a base for determining probabilities of detecting various infestation levels as increasing numbers of samples are collected. Such a method saves time, labor and money in the detection and ensures the relative proportional certainty emanating from the inspected sample to the actual matter. However, a zero tolerance is required for complete eradication of the disease. Neither Poisson nor the negative binomial distributions can be applied to such a zero tolerance. Eventually, the tolerance limit must be backed up by sound technical information and consequent judgments for each specific case.

Key words: Quarantine · Integrated management · *Ralstonia solanacearum*

INTRODUCTION

Root and tuber crops are the most important food commodities produced in many subtropical and tropical countries. World production figures show that root and tuber cultivation is increasing and the rich sources of carbohydrates in the tropical and subtropical regoin, where clonal reproduction and poor soils give those advantages for subsistence agricultures and are occupied the level second to cereals in total world supply[1]. Yet, two of these crops have somewhat similar Arabic pronunciation with consequent confusion: the solanum potato (*Solanum tuberosum*) and the sweet potato (*Ipomoea batata*). The selenium or Irish potato, originated from the Andean highlands of South America, is a major food crop in 57 countries, which is more countries than any other single crop, with the exception of maize and it is the only tuber crop produced in any significant amount in the developed countries. It ranks first and third in the list of edible energy and protein production per hectare per day, respectively [2].

In recent years, in subtropical and tropical countries, potato production has spread gradually out of its traditionally cool environment at higher altitudes into

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hotter and, generally, drier areas. It is increasingly grown as a winter crop in many irrigated, arid areas of large, commercial farms as better varieties have become available in developing countries. Eventually, the production of this crop has also been expanding to relatively warm and humid zones that are optimum for the development of many pathogens and pests.

The Importance of Potato in Egypt and Threats of Potato Pests and Pathogens: In Egypt, potato has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato. In addition, total cultivation of potatoes reached 197.250 feddans (4200 m²) which produced 2,039,350 tons of tubers with an average yield of 10.34 tons/Feddan [3]. This crop is economically important to Egypt and any disturbance in its production affects severely its local and more importantly export impact. During their seasonal plantations (Summer, Nili and Fall), potato plants are subjected to numerous pathogens and insect pests which cause considerable loss in Egyptian quantitative and qualitative potato yield. Such pathogenic and insect problems include the fungal pathogens (and their diseases): *Alternaria solani* (early blight) and *Phytophthora infestans* (late blight); bacterial pathogens: *Ralstonia* (*Pseudomonas*) *solanacearum* (brown rot or bacterial wilt), *Erwinia carotovora* sub. sp. *atroseptica* (black leg and rot Erwinia), *Clavibacter michiganense*. sub. sp. *sepedonicum* (ring rot) and *Streptomyces scabies* (common scob); nematode diseases especially those caused by *Meloidogyne incognita*, *M. javanica* and *M. arenaria ;* virus diseases: mottle or latent virus (*Marmor dubium* var. *annulus*), mild mosaic (*Marmor solani*), Potato virus (Y) or severe mosaic (*M. cucumeris upsilon*) and potato leaf-roll virus (*Corium solani*) and insect such as *Gryllotalpa gryllotalpa*, *Agrotis ipselon*, *Pentodon lispinosus*, *Agrypnus notodonalas*, *Myzzus lersicae*, *Phthorimaea operclella*, *Spodaptera littoralis*, *Euzophora asseatella*, *Empoasca discipiens*, *Bemisia tabaci* and *Liromyza begoniae*. Thus, a key barrier to the improvement of potato in Egypt is the reduction in yield and tuber quality caused mainly by potato pests and pathogens [4,5].

The Potato Brown Rot Danger in Egypt

The Causal Pathogen of Brown Rot: The disease was first recorded on potato in Egypt, by Brition-Jones [6]. However, the causal bacterium was isolated for the first time in Egypt from potato tubers showing brown rot symptoms by Sabet [7]. In Germany, Gehring [8] isolated *Pseudomonas solanacearum* (Smith) Smith from imported Egyptian potato tubers showing brown discoloration of the vascular ring. The pathogen nomenclature was changed successively from time to time ended by *Ralstonia solanacerum* named by Yabuachi *et al.* [9] who suggested the name to the causal organism instead of *P. solanacearum.* Full description of this disease on potato and tomato was given by Kelman [10]. *R. solanacearum* is a strictly aerobic, non-spore forming, Gram-negative organism, with a wide and diverse host range affecting several hundred plant species from 44 families, including the Solanaceae, Compositae and Leguminosae. Host plants of economic importance include potato, tomato, tobacco, pepper, eggplant, groundnut and banana. In addition, several ornamental plants and weeds can act as host reservoirs of infection. *R. solanacearum* is very complex and highly variable. Strains of *R. solanacearum* are grouped into five races according to the host or hosts primarily affected and five biovars according to the use of selected biochemical properties [11]. Of the five races, 1 and 3 cause symptoms on potato, with major yield losses from rotting tubers (brown rot) and wilting with subsequent death of the plant (bacterial wilt). Race 3 is adapted to pathogenesis at lower ambient temperatures and is believed to have originated in the temperate highlands of Peru and Bolivia. It is closely associated with the potato and is responsible for the present brown rot outbreaks in Europe and North Africa [12]. Although *Ralstonia solanacearum* (race 3) is a soil borne pathogen that persists in wet soils, deep soil layers (75 cm) and reservoir plants [13], its distribution in potato fields can be spotty and is commonly found in areas that have poor drainage [14]. Bacterial wilt on tomato caused by *Ralstonia solanacearum* (race 1, biovar 1) causes wilt by infecting plants through roots and colonizing stem vascular tissue. Infection of the potato plant commonly occurs via the soil, where bacteria enter the root system of the plant at root emergence points, at wound sites, *e.g.* caused by nematode activity or soil particle abrasion, or via infected mother tubers. The pathogen has a quarantine status in the European Union (EU) to restrict its spread, as infections can be very destructive and cause considerable yield losses.

The Disease Symptoms: The pathogen enters the vascular system of the plant and under favorable conditions cell numbers increase and spread up the stem and to tubers. In warmer regions, where transpiration rates are high, the disease usually manifests itself as a general wilting of the shoot system (bacterial wilt). In cooler regions, wilting may be less evident or absent. Where symptoms develop, brown staining is seen in the tissues of cutted tubers, caused by cell-wall-degrading enzymes produced by the bacteria. Bacterial ooze may also exude from the potato and, in severe cases, from the eyes resulting in soil sticking to the exterior of the tubers. As disease progresses a general rot may develop. Tubers may also harbor latent infections . Thus, although the bacterium can cause wilting of the potato plant, the symptoms you are most likely to see are in the tuber. Wilting of the leaves starts towards the top of the plant and may be initially confined to one side of a leaf or to only one stem. This can lead to rapid drying and death of the whole plant. Contrary to Egyptian potato plants, wilting has rarely been observed in European crops. Symptom expression occurs at different rates in different varieties and is favored by warm temperatures (above 15°C with optimum around 25°C) and other environmental conditions (especially high soil moisture). When the bacteria can latently infect tubers without causing noticeable symptoms, the pathogen can survive in seed tubers during storage and cause disease at planting in the next season [15].

The Spread Methods and Survival of *Ralstonia solanacerum*: The most effective means of spread of brown rot worldwide is through distribution and planting of infected seed potatoes. *R. solanacerum*-infected plant material has always been regarded as the greatest risk for both short- and long-distance dispersal of *R. solanacearum* race 3 in Egypt and on a global scale. Although seed transmission has occurred in some European countries, all UK potato cases were due to water-logging following irrigation, or flooding from contaminated watercourses [12]. In almost all European outbreaks associated with contaminated water, woody nightshade (*Solanum dulcamara*) plants with roots growing in contaminated watercourses have been found to be infected and this has acted as a continuing source of infection in the water. It is likely that contamination of watercourses has occurred in the past through discharge of untreated waste from imported infected ware potatoes. The bacterium can also survive from season to another in potato groundkeepers (unharvested potatoes from the previous crop). Spread may also occur via contaminated equipment or from waste dumps containing contaminated material. Resistance, or more accurately tolerance, to the disease has been observed in potato. A major source of resistance to *R. solanacearum* has been came from *S. phureja* cv. but resistance genes from other potato species, including *S. tuberosum*, have been identified and used. Tolerance, in potatoes that harbor relatively large populations of *R. solanacearum* in the absence of disease symptoms, increases the risk of spread through trading infected tubers. In addition, potato cultivars carrying resistance to bacterial wilt, *R. solanacearum*, lose their resistance when planted in *Meloidogyne*-infested soil [16]. Eventually, the disease can be spread by trade in ware potatoes, via irrigation water, plant debris, mechanical transmission, insects, root-to-root transmission, wind and rain, wounding during cultivation practices and by nematodes.

The survival of *R. solanacearum* race 3 in the environment is not well understood. However, protecting the organism from desiccation and antagonism by other microorganisms can prolong this survival. *R. solanacearum* tends to persist longer in wet but well-drained soil, in the deeper soil layers (>75 cm), or in the presence of alternative crops, weed hosts or groundkeepers. Survival in soil is reduced by extreme cold and the presence of antagonistic microorganisms [12]. *R. solanacearum* infects alternative hosts including solanaceous weeds, which increases the likelihood of survival and spread. Although disease symptoms may not develop in these hosts, bacteria continue to multiply and may become a source for re-infection of potatoes. Race 3 has been shown to survive and multiply in the roots of the secondary hosts Bittersweet (*Solanum dulcamara*) and Deadly Nightshade (*Solanum nigrum*) growing in rivers in a number of European countries [12].This survival in secondary hosts, as well as in groundkeepers, could make crop rotation ineffective.

The Brown Rot Disease and the Problem of Exports to the European Union: In Egypt, potato plants are considered on of the most important hosts of *Ralstonia solanacerum*. In addition to considerable qualitative and quantitative yield losses caused by the brown rot disease, the existence of *R. solanacerum* in soil hinders the cultivation of potato, in such a soil, for the production of seed tubers or exportation. As *R. solanacerum* is a quarantine organism there can be large costs due to disease testing and administration of seed production to control the disease. In March and April of 1996 France, Finland, Spain and Denmark banned imports of potatoes from Egypt on the basis of continued interceptions of potato containing *R. solanacearum*. By Decision 96/301/EC of May 3, 1996 the European Commission (EC) imposed a series of "additional restrictions" on imports of potatoes from Egypt. Russell [12] reported the "additional restrictions" which required that in order to be imported into the EU, each "lot" of Egyptian potatoes (or every 25 tons) coming from a "qualified area" (areas certified by Egypt as areas in which *R. solanacearum* is not known to occur) must be:

1) sampled (at least 200 tubers) immediately prior to shipment and found to be free from any symptoms of brown potato rot; 2) tested for the latent infection with *R. solanacearum* of each consignment with one sample for each "area" represented in the consignment with a minimum in any case of five samples; 3) harvested, handled and bagged separately "area by area" including "reasonably separate" use of machinery; 4) prepared in "lots" composed of potatoes harvested in one single area; 5) labeled on each bag with the relevant code for each "qualified area" and each lot number; and 6) accompanied by an official Egyptian phytosanitary certificate indicating lot number and "qualified area" code number as well as an official statement that the required testing has been conducted.

In addition, the EU designated authorized points of entry and officials in charge of entries of the potatoes. These officials were to be notified in advance of each entry as to likely time of arrival and amount. Potatoes arriving at the designated port of entry must then be inspected and tested by lot (at least as in 1 and 2 above). The lots must remain separate and could not be marketed or used until "it has been established that the presence of *R. solanacearum* was not suspected or detected". The list of "qualified areas" could then be adjusted by the Commission based on results of these procedures. Finally, the potatoes were to be labeled to prevent them from being planted.

In the 1996/97 potato import season there were "considerable numbers" of Egyptian potatoes intercepted with brown rot. In response the EC strengthened the provisions of 96/301/EC. By Decision 98/105/EC the Commission banned Egyptian potato imports unless they met more stringent requirements which now included provisions that:

1) potatoes destined for the EU be produced in fields located in "qualified areas" declared by Egypt to have never any outbreak of *R. solanacearum*; 2) the qualified areas must be tested for the bacteria and found free of it prior to planting; 3) potatoes must be inspected in the field for brown rot and found free from any symptoms in laboratory tests; 4) potatoes must be accompanied by documentation at the packing station stating their origin by area; 5) potatoes must be sampled at the packing station for symptoms of brown rot with 10% of 70kg sacks to be inspected and 50% of larger sacks (at least 40 potatoes sampled per sack); 6) 2% of sacks must be sampled after packing and found free of symptoms; 7) at least one sample per area in each consignment must be tested under a EU authorized test for latent infection; 8) potatoes must be exported by an officially registered exporter; 9) where "typical or suspect" symptoms of brown rot are detected at the port of entry all lots in the same consignment from the same area to be held until presence of *R. solanacearum* is "refuted" by approved EU tests; and 10) testing at the point of entry for latent infection also must be done on samples from each area and the potatoes shall be held until it is established that presence of the bacteria was not confirmed.

During the 1997/98 import season Finland and Denmark banned imports of Egyptian potatoes. On August 11, 1998 the Commission responded to continued complaints of brown rot shipments by adopting new measures in Decision 98/503/EC. In this Decision the Commission eliminated the concept of "qualified areas" (those in which outbreak of *R. solanacearum* was not known to occurred) and replaced it with "pest free area" (an area in which such an outbreak was known not to occurred). Such areas were to be designated in accordance with UN Food and Agriculture Organization (FAO) standards. No imports of potatoes were allowed which did not come from these certified "pest free areas". Egyptian potatoes imported into the EU were also to be grown from potatoes directly of EU origin or "once grown from such potatoes, produced in an approved pest free area" tested for latent infection immediately prior to planting". Additional certification requirements were also imposed on packing stations, most notably that stations used for packing potatoes for export to the EU must handle only potatoes eligible for EU export and no others. Even imports from "pest free areas" would be banned if more than 5 interceptions of *R. solanacearum* were found in lots imported into the EU during the season. Article 1.3 of Decision 98/503 is the provision that provides for the cutting off of shipment after 5 interceptions; this article does not discuss the basis for choosing the number five (Russell, 2008). Decision 98/503 and the other EC decisions also do not discuss the extent to which measures provided for are based on risk assessments. On 22 September 1998, the EU, under the provision for notification of Article 7 and Annex B of the SPS Agreement, notified the World Trade Organization (WTO) of the implementation of the Commission Decision 98/503/EC as an emergency measure (G/SPS/N/EEC/63).

In the 1998/99 import season there were again a "number of interceptions" of *P.S.* (Smith) infected potatoes from Egypt and imports of Egyptian potatoes were prohibited on April 3, 1999. As a result of this ban Egypt further strengthened its harvesting, handling and packing regime administered by the central administration for plant quarantine. Egypt also improved measures taken against exporters violating EU potato export regulations and adopted a new more tightly regulated control system for "pest free areas" (see Ministerial Decrees No. 61 and 95 of 2000 and No. 1317 of 2001). Based on these changes and assurances, on November 30, 1999 the EC issued Decision 1999/842/EC which re-allowed imports of Egyptian potatoes from designated "pest free areas" on essentially the same conditions as in 98/503/EC.

In the 1999/2000 potato season only one interception of *R. solanacearum* was found in Egyptian potato exports to the EU. By Decision 2000/568/EC of 8 September 2000 the Commission allowed continued imports from "pest free areas" on the same terms as in 1999/842/EC.

During the 2000/2001 season "a number" of interceptions of *R. solanacearum* occurred and Egyptian potato imports were again banned on May 5, 2001. The EU reassessed its position and obtained new assurances from Egypt of strict control measures within "pest free areas" and confirmation of measures against exporters who violated regulations on EU potato exports. In addition, Egypt submitted a detailed contingency plan explaining measures applied when brown rot is found within Egypt or in consignments of Egyptian potatoes at EU entry points. Based on this information, the EU allowed imports of potatoes in the 2001/2002 season from designated "pest free areas" in Egypt on the same substantive terms as contained in Decision 2000/568/EC. Since then, yearly renovation of the system is adopted where the decision to open the EU market to Egyptian potatoes coming from approved Pest Free Areas (PFAs) is taken by Member States in the Standing Committee on Plant Health (SCPH) held at the end of September every year in Brussels. Prior to this meeting, COM (DG SANCO E.1) organizes an ad hoc working group at the beginning of September to examine the information supplied by Egypt on their investigations on interceptions of Egyptian potatoes contaminated with Brown Rot during the last season and Egypt's proposed list of PFAs for the coming season. Upon a suggestion of COM (DG SANCO E.1) and discussions on information provided by Egypt, the SCPH takes the decision to re-open (or not) the export possibility of Egyptian potatoes originating in the approved PFAs. In addition, the SCPH can discuss/approve any amendment to the provisions governing the regime. COM then undertakes the necessary legal drafting in order to re-open the possibility for Egypt to export potatoes to the EU in the next season and presents the text in one of the forthcoming meetings of the SCPH (usually end October). The permitted potato export season for Egypt usually lasts from January to May/June. Finally the Commission Decision allowing the entry into the EU of potatoes from PFAs of Egypt is usually adopted and published in the Official Journal (OJ) by the end of the year. The Commission Decision 2004/836/EC, amending Commission Decision 2004/4/EC on the import of Egyptian potatoes during the import season 2004/2005 was adopted on 6 December 2004 and published in the OJ L 360 (7.12.2004). Unfortunately, following last export season high number of Brown Rot-infected tubers was found in Egyptian potatoes exported to the EU, the Egyptian Ministry of Agriculture and the Ministry of Foreign Trade and Industry have jointly issued Decree 757/2005 establishing more strict rules for the production, preparation, examination and exportation of potatoes for the season 2005/2006 and thereafter. Latest Egyptian legislation governing the PFA system was formulated according to the Decree in which exportation of potatoes was only allowed to those companies that exported an amount not less than 4000 tons to the EU during last season and limited to a quantity not exceeding those that were exported last season. Companies and packing stations will be suspended for the rest of the season if proven their responsibility on the infection of exported potatoes. More severe procedures for the preparation of potatoes have been also adopted in particular on the monitoring and control of the packing stations. In addition, the Ministry of Foreign Trade and Industry has suspended a number of companies and packing stations from exporting/operating potatoes to be exported to the EU for one year due to their negative record on interceptions during last export season. The complete list of companies and packing stations has been published in MFTI Decree 507/2005. It is noteworthy that the total value of Egyptian potato exports fell from a peak value of US$ 102.12 million in 1995 to $US 7.7 million in 2000 [12].Although 2000 exports may be unusually low, exports were over US$40 million in both 1998 and 1999, even these numbers are a 56% decline over the period. This represents a drop in tonnage from approximately 419,000 metric tons to 48,500 tons. Potato exports to the EU followed a similar pattern falling to a near decade low of 110,000 tons in 1999/2000 down from a peak of over 300,000 tons in 1995/96.

On the other hand, the core of the Association Agreement for EU-Egypt trade is the establishment of a

Free Trade Area between the EU and Egypt, which implies reciprocal tariff liberalization for industrial and agricultural goods. The tariff dismantling provisions are asymmetrical in favor of Egypt as a developing Mediterranean country. The EU has granted a complete dismantling of customs duties and quotas for Egyptian industrial products and a list of agricultural products exported to the EU. Also, Egypt is implementing a gradual abolition of customs duties for European industrial products and some agricultural products over an implementation period of 14 years. Consequently, EU-Egypt bilateral trade has been steadily increasing: €11,5 billion in 2004, €13,3 billion in 2005 and € 16.3 billion in 2006 (a 63% increase compared to an average of € 10 billion before implementation of the Agreement) with an upward trend for both Egyptian exports to the EU which have increased by 45% in 2006 and EU exports to Egypt which increased by 6%. Egypt's trade deficit has reduced significantly as exports increased: € 1.2 bi in 2006 compared to € 3.2 bi in 2004. The enlarged EU is the first trade partner for Egypt and represents about 40% of Egypt's total trade with the world [17].

European and Egyptian Cooperation and View of Exported Potatoes: Since 1992 over ten EU inspection teams have visited Egypt regarding potato brown rot. The EU has contributed substantial resources to the Potato Brown Rot Project and the development of plant quarantine capacity in Egypt. Egyptian farmers, packers and exporters of potatoes have raised sustained and increasing concern about the EU requirements, delays in identifying PFAs and discriminatory treatment. These concerns have been expressed at the highest levels by individual businessmen and business organizations. Some have also questioned the basis for banning exports after five shipments and asked whether the EU considered other measures and inspection procedures that were less time consuming, produced less uncertainty and had a smaller impact on the volume of potato exports to the EU. Some have also asked about the effectiveness of the measures to control Brown Rot in the Netherlands and whether these measures are still in effect or necessary [12].On the other hand, the European Commission (EC) authorized Egyptian potato exports to the EU for the next export season during the Plant Health Standing Committee's (PHSC) recent meeting in Brussels. The committee's approval signals Egyptian compliance with EU phytosanitary standards and accords with the trade preferences granted to Egypt under the Egypt-EU Association Agreement. Egypt, together with the EU, established the Potato Brown Rot Project (PBRP). The project set up laboratories to test potatoes and identified a number of PFAs. It has trained local staff and formulated a traceability and report system for brown rot, in addition to providing advice on disease control and undertaking research on the causes of brown rot in Egypt. After eight years work in the project, funded by a 2.6 million euro EU grant and a further two million euros from Egypt, has succeeded in bringing down brown rot infestation rates from 17 percent to 1.7 percent, (El-Haddad, head of PBRP, personal comm.). Given such a success, potato exports to the EU have at last started to rise. Despite recent increases, total potato exports to the EU remain well below 400000 tons of table potatoes recorded before the outbreaks of brown rot disease [18]. While the EU continues to stop Egyptian potatoes at its borders because of the disease, many Egyptian exporters believe that European fears are unfounded. They stress that brown rot is harmless to humans and only affects the soil and that Egypt exports table not seed potatoes. Giving the Egyptian exporters' argument its due weight, one can not neglect seeing the implication in the European side, i.e. if by mistake such a disease is to be spread into other crops and soil in Europe, all measures to be implemented will be less effective. It will also necessitate large investment on tackling the disease control with feeble results. Therefore, it is an issue of export survival to do out with the disease before exportation for the benefit of both Egyptian and European sides.

A British scientist Russell, [12] wondered why EU provision insists on finding neither more nor less than five interceptions of *R. solanacearum* in the imported tubers in order to reject the shipment. Some Egyptian exporters have also questioned the basis for banning exports after five interceptions of Brown Rot in potatoes originating in Egypt without discussing the basis for choosing the number five. Such a wonder could be scientifically analyzed through a statistical approach [19]. Using such an approach for quarantine problems, probabilities of detecting an infested unit of nematode [20] and insect [21] pest infestations were computed based on the binomial distribution. Similarly, we presented (Table 1) probabilities of detecting an infested potato tuber for six levels of *R. solanacearum* infestation. We used the binomial because it is more conservative than the hypergeometric distribution and therefore the lot size in the latter distribution should not be large relative to the sample size. Also, it is noteworthy that the tolerance limit for each sampling test should depend on the economic importance in terms of destructive potential of the pest

Table 1: Probability of detection based on binomial distribution at each of six levels of *Ralstonia solanacearum* infection using selected potato tuber numbers as samples per lot

Lot size (L)	Number of samples(n)	Probability of detecting infested potato tubers if the lot sampled is 1% infested	5% infested	15% infested	25% infested	40% infested	50% infested
70	5	0.969	0.922	0.763	0.556	0.226	0.049
70	10	>0.999	0.994	0.944	0.803	0.401	0.096
70	30	>0.999	>0.999	>0.999	0.992	0.785	0.260
100	10	>0.999	0.994	0.944	0.803	0.401	0.096
100	30	>0.999	>0.999	>0.999	0.992	0.785	0.260
100	50	>0.999	>0.999	>0.999	>0.999	0.923	0.395
200	50	>0.999	>0.999	>0.999	>0.999	0.923	0.395
200	70	>0.999	>0.999	>0.999	>0.999	0.972	0.505
200	85	>0.999	>0.999	>0.999	>0.999	0.987	0.574
500	50	>0.999	>0.999	>0.999	>0.999	0.923	0.395
500	100	>0.999	>0.999	>0.999	>0.999	0.994	0.634
500	200	>0.999	>0.999	>0.999	>0.999	>0.999	0.866
1000	200	>0.999	>0.999	>0.999	>0.999	>0.999	0.866
1000	400	>0.999	>0.999	>0.999	>0.999	>0.999	0.982
1000	500	>0.999	>0.999	>0.999	>0.999	>0.999	0.993
10000	10	>0.999	0.994	0.944	0.803	0.401	0.096
10000	100	>0.999	>0.999	>0.999	>0.999	0.994	0.634
10000	1000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999

species, the relative sampling error, inspector and quarantine regulations. Basically, unless funds are available to sample every seedling or unit, zero tolerance limit will not be an acceptable level. Clearly, probabilities of detection must be weighed against the cost of the additional labor require [20]. In addition to enhancing sample size, detection may be improved through different schemes. Maas [22]suggested changing the common sampling pattern to non-random sampling directed at plants showing symptoms of inspected diseases. Abd-Elgawad [23] employed three sampling patterns to investigate sample size optimization needed to achieve a predetermined level of sampling error for several plant-parasitic nematode species. Admittedly, because of the wide variety of crops, pests, pathogens and their distributions, it is unlikely that any one sampling plan will suffice in all situations [24]. Nevertheless, sound and technical information on quarantine organisms which deal with the organism's recognition, known distribution, biology and main means of spread are fundamentals in order to evaluate the potential of such alien species in terms of whether its infections can be very destructive and cause considerable yield losses and also to produce a cost/benefit analysis of control by phytosanitary regulation as opposed to 'living with the pest' [25]. Moreover, quarantine procedures which deal with methods of detection as well as specific quarantine requirements which deal with measures to reduce the risk of disease spread must be provided.

Ambitious Plan for Eradicating the Disease from Egypt: Given the problems that have occurred especially in potato and tomato production which have been identified as the key crops at risk, Egypt must take measures to locate *R. solanacearum*, prevent its occurrence and spread and control it with the aim of suppressing its population to near zero level or eradication. Such measures must include several integrated management approaches:

Legislative and Cultural Measures: These measures must be taken both directly on tomato and potato crops and in relation to the risk of spread of the pathogen from other host crops or situations (*e.g.*, water/weed infestations) onto these two crops. The hosts should be considered as plants (including tubers), other than true seed of potato and plants other than fruits and seeds of tomato. True seed has been excluded from the scope since the risk of spread of the pathogen in true seed has been considered negligible and tomato fruit has been excluded because the risk of contamination of fruit is considered a very lower risk compared to the risks posed by plants (including potato tubers) of potato and tomato.

The organism is listed as a quarantine species at EU without specification of race or biovar thus ensuring that any finding of this organism whether race 1 or 3 , or any other race/biovar, has to be considered for action under their control Directive. Such an approach should be followed in Egypt considering the following (similar) steps:

Surveys: In order to locate *R.. solanacearum*, the CAPQ is required to conduct annual systematic official surveys on potato and tomato crops, the latter focused on young tomato transplant crops. Also, in order to identify other possible sources of contamination threatening potato and tomato production and according to the risk identified e.g. from imports or from positive findings of *R. solanacearum* in an area, targeted official surveys are required on other host plants, solanaceous weeds and water/ liquid waste posing a risk for waterborne spread.

Reporting: There should be a general reporting of the suspected occurrence or confirmed presence of *R. solanacearum* irrespective of crop or other situation in which it has been found on a regular basis. If it is found in tomato and potato crops or it is likely to affect such production (*e.g*. by irrigation), action has to be taken under the proposed control Directive (the CAPQ). If it has been found in other crops or situations with no likely affect on tomato and potato production, action to destroy the pathogen, irrespective of the race/biovar, has to be taken under the CAPQ supervision.

Action: Once a report has been received, the CAPQ have to follow a Community test method to confirm or refute the presence of *R. solanacearum*. Whilst the report is still "suspect" with no final confirmation of the pathogen measures to prevent movement of suspect material as well as initial steps to trace the origin of the outbreak have to be taken. Once the report is confirmed, the following steps to identify and delimit the outbreak must be taken immediately:

1. Tracing of the origin of the outbreak,
2. Designation of contamination including potato tubers, tomato and potato plants, fields and places of production,
3. Determination of the extent of probable contamination through pre- or post-harvest contact, through production, irrigation or spraying links or through clonal relationship with the designated contamination,
4. Demarcation of a zone, taking into account factors posing a risk for the spread of the organism, including the risks of soil and water borne spread. The latter should be particularly considered in relation to irrigation or spraying of crops using contaminated water.

These steps apply not only to outbreaks in tomato and potato crops but also to other host crops and to surface water found to be contaminated when tomato and potato production is or has been at risk. For surface water this involves a designation as contaminated of the river or water course involved so that appropriate controls can be introduced, e.g. a ban on use of the water for irrigation of the host crops.

In Egypt, legislative measures do exist but are not always put into effect. Inspection of plants especially for *R. solanacearum* is not seriously undertaken. Quarantine measures should be enforced to prevent the transport of any infected plant parts, infected soil, or organic manures to newly reclaimed areas [26]. Control measures should be taken as follows:

1. Contaminated plant material (e.g. potato tubers) must be destroyed or subjected to strict measures e.g. incineration, deep burial or processing with very stringent waste disposal conditions. Probably contaminated plant material (e.g. potato tubers) must not be planted but can be used for human consumption with strict controls on packaging or processing and on waste disposal,
2. Contaminated or probably contaminated machinery, stores, packaging etc must be either destroyed or decontaminated by cleansing and/or disinfection.
3. In the demarcated zone, a series of control measures have to be imposed over a period of time:

(i) on contaminated places of production because of the risk of survival of the pathogen in the soil, weed hosts or volunteer plants, strict measures include:

- Surveying for and elimination of weed hosts and volunteer plants
- Planting restrictions over a number of years. These planting restrictions are strictest on contaminated fields *e.g.* a minimum four year prohibition on planting potato, tomato or other host plants and

crops for which there is an identified risk of the organism spreading or surviving,

- Planting of certified potato seed only,
- Introduction of a crop rotation cycle before production of seed tubers,
- General survey of all potato and tomato crops (including testing of potato crops),
- Cleansing of machinery and storage facilities involved in potato or tomato production,
- Official controls (including a ban) on irrigation and spraying programs, so as to prevent the spread of the organism.

(ii) on other places of production, for at least the three growing years after the disease outbreak, a series of control measures are laid down:

- Overall official supervision of potato and tomato production,
- Cleansing of machinery and stores used in such production,
- A requirement for planting only of certified seed or seed grown under official control,
- Separate handling of seed to ware stocks,
- Official survey of potato and tomato crops,
- General requirement for progressive replacement of seed potato stocks,
- In cases where surface water is contaminated, a specific annual survey on surface water and associated *solanaceous* host plants and official controls on irrigation and drainage regimes as well as controls on waste disposal from processing premises. Eventually, all useful cultural practices should be followed, i.e using resistant varieties, crop rotation, alteration of cultural practices and selection of free disease planting parts or seeds. Previous outbreaks in Europe and the United States were controlled through good sanitation measures similar to the above-mentioned suggestions.

Chemical Control: Chemical control is often carried out by soil fumigation or antibiotics treatment (streptomycin, ampicillin, tetracycline and penicillin). Yet, using chemicals and antibiotics became a routine practice to control plant diseases which showed by time, little effect against majority of bacterial plant pathogens [27,28].

Biological Control: Recently, biological control of plant pests and pathogens are used with a clear aim at the avoidance of environmental pollution. Microorganisms that can grow in the rhizosphere are ideal to be used as biocontrol agents, since the rhizosphere provides the front-line defense for roots against attack by pathogens. Although pathogen-suppressive microorganisms in soils are rare, those identified as excellent examples of the full potential of biocontrol of soil borne pathogens. Bacteria shown to have potential biocontrol action occur in many genera and species such as *Erwinia* spp., *Pseudomonas fluorescens*, *P. syringae*. Biocontrol agents are not limited to a specific bacterial group. However, given the diversity of the rhizosphere microflora, it is probable that the full spectrum of potentially effective strains has barely been explored. Nevertheless, the chance of selecting effective strains may be improved initially by isolating bacteria from the same environment in which they be used. Such a procedure was implemented in Egyptian potato fields infested by the brown rot disease and eleven bacterial isolates proved to produce antagonistic substances against the indicator bacterium *R. solanacearum* [11]. Characterization of the antagonistic substances produced by the eleven isolates revealed that three of them (B6, B9, B11) are non-heat labile and protease sensitive. These two criteria indicated that the previous isolates are bacteriocin producers. Isolate B11 was chosen for its superior antagonistic activity compared to B6 and B9 against *R. solanacearum*, where B11 isolate was designated as S2HA. Optimizing bacteriocin production revealed that, optimum production/activity can be obtained after 48 h incubation period in Beef–Peptone medium at pH 7, where the medium was supplemented with 2% glucose and the producer isolate S2HA grew at 30 oC. The preliminary identification of the bacteriocin producer isolate S2HA indicated that it is a member of Enterobacteriaceae. The bacteriological identification tests revealed that S2HA is a motile short rod cell, gram negative, positive for catalase, while it was negative for oxidase, indole, urease, nitrate, citrate, VP and MR. It was fermenting to glucose and non-fermenting for sucrose, lactose and glycerol. The previous results indicate that S2HA can be identified as a species of *Pantoea* sp. (previously *Erwinia herbicola*). Confirmatory test based on 16S rRNA phylogeny and the sequencing of 349 bp fragment assigned the S2HA isolate as *Pantoea* sp. with more than 88% relative phylogeny.

The Molecular weight of the purified bacteriocin was determined by SDS polyacrylamide gel electrophoresis revealing one band with a molecular weight of 29,000 daltons in relation to the molecular weight of the marker and the relative mobility of the band. The biocine S2HA found to contain 259 amino acids species with molar percentage ranging from 0.31 to 23.09% for "*pro*" to

Fig. 1A: Both fields are infested with *Ralstonia solanacerum*

Fig. 1B: Biocine-treated potato field compared to untreated field

"*ileu",* respectively. The biocine S2HA molecular mass was determined from the sum of the molecular mass of each residue multiplied by their respective number in the Bioicine S2HA. It was found to have a molecular mass of about 30,675 daltons.

Purified bacteriocin was diluted to determine the minimal inhibitory concentration (MIC). The MIC considers as an indicator for the specific activity of the bacteriocin, where the smaller MIC that exhibits inhibitory affect the greater specific activity of the purified bacteriocin. The purified bacteriocin of S2HA showed a dilution factor of 16 folds producing positive inhibitory effect against the indicator strain *R. solanacearum*.

The production/activity of bacteriocin in liquid medium was attributed to the growth phases of bacterial cells in the growing culture. The bacterial isolate S2HA reached early log phase with bacteriocin activity at AU= 9 after 4 h of bacterial inoculation. It is gradually increased by incubation time until reached its maximum activity (AU=64) at 14 h (late log phase/early stationary phase). The bacteriocin activity was maintained at its maximum activity (AU=64) for 2 h. A gradual decrease in activity by time was observed in correspondence to the protein concentration, which was decreased from 1.8 mg/ml at 14 h by time to its minimum 0.28 mg/ml after 24 h.

The results of *in planta* assay indicated that plants infected with *R. solanacearum* led to almost 93% death, while untreated plants or plants treated with only Biocine S2HA remained healthy at 100 and 96.7%, respectively (Fig. 1A and B). Plants treated once with both *R. solanacearum* SO2 and Biocine S2HA did not show any significant control, where it produced 86.7% plant death. The infected plants that were weekly irrigated with Biocine S2HA at different concentrations showed variable levels of control. The dilutions 1/4, 1/8, 1/16, 1/32 of Biocine S2HA produced significant disease control leading to healthy plants of about 96.7%, 90.0%, 70.0%, 26.7%, respectively.

Yet, effective and commercial use of bacteriocins requires consideration of many questions, including assessment of disease severity, availability and effectiveness of suitable bacteriocin, sensitivity of a majority of test strains, compatibility of bacteriocin mixture, useful lifetimes, potential additives to maintain or prolong biological activity and inducing maximum yields. Eventually, our team is following up the procedure required for the development of a successful biological control agent which involves initial selection of a suitable antagonist by laboratory and small-scale field testing, the formulation of an effective strategy of application, (including both timing and method of biocine application) and finally carry out a large-scale field trials to establish the biological and cost-effectiveness of control under agricultural conditions.

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