Epidemiology and distribution of soil borne viruses in the fields of potato, and are disseminations with out via-vectors

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Abstract. Through visits farm of potato fields in the area of Rashidiya- of Nineveh province (Iraq) and the container on the viruses symptoms, collect soil samples from areas near the root zone of plants that those samples contained two viruses Tobacco mosaic virus, it was detected only in soil and Potato virus X, was detected in the infected plants debris, which has been detected them biological by using indicator plants show local lesions and by immunologically test DAS-ELISA, did not notice any living vector escort through the examination of soil samples.

1. Introduction:

A large number of soil-borne viruses have been recorded in recent years that are spread without need for biotic vectors, have been discovered in soil and groundwater in addition to the main water sources as rivers, lakes and seas. There are more than 50 viruses belong to 12 families of soil-borne viruses indicated by (Verchot-Lubiez- 2003). Two soil-borne viruses groups have been identified long time ago that transmitted by biotic vectors, where first group is transmitted through Nematode which belongs to species of Trichodorus, Longidorus and Xiphinima, viruses of this group are called (NEPO and TOBRO- viruses), while the other group is transmitted by zoo spores of soil-habitat fungi that belong to species of (Olpidium, Spongospora and Polymyxa) that called (FURO and BYMO viruses).

At present, a new group of soil-borne viruses can be added which is characterized by being existent in soil and water in the form of viral particle (virions) that can move and transport from infected plants to other uninfected ones without biotic vectors mentioned previously. A number of characteristics of this group were summarized by (Koeing, 1986): can’t be transported by aerobic vectors (arthropods, flying insects as aphids and white flies ..etc.), reaches up to high concentrations among its host plant, infects the plants in the surrounding soil through the roots, high constancy within living cells (whether plants or debris alike), its capability to infect its host plant through the roots without need for biotic vectors, wide host range, owning simple group of genes, and a large part of this group can be translated mechanically.

Since many of them are considered dangerous viral pathogen that inflict tangible economic losses on agricultural crop, to name just a few, Beet necrotic yellow vein virus(BNYVV), Cucumber green mottle mosaic virus(CGMMV), Tobacco rattle virus (TRV), Tomato bushy stunt virus(TBSV) and Tomato mosaic virus(TMV) (Sparr,1993). Consequently, it has become necessary conducting a
comprehensive study to figure out what these viruses are, rules of their spread in the nature, determine the characteristics of soil that impact them, as well as developing new methods to isolate and detect them in sample of soil and water to assist place appropriate programs to prevent them from proliferation.

2. Material and methods of action:

Samples collection:

During the field visit to Potato farms in Al-Rashidiya district of Nineveh city, unusual symptoms like mosaic and stunting had been observed as illustrated in (Figure 1). Therefore, samples were gathered of both fall yield (mid of January, 2013) and spring yield (early March), and marking the infected areas to collect soil samples later.

Soil samples collection:

Samples of soil suspected to be contaminated had been collected off the place surrounding the roots of plants uprooted by 15cm deep and of 10 cm wide during flowering phase. Targeted samples had been kept in polyethylene bags before brought to the laboratory to extract and detect viruses.

![Figure (1): Symptoms of stunting and mosaic on potato samples](image)

Preparation of polluted (infected) soil for biological and serological tests:

A one gram of polluted soil was taken and mixed with 1ml of phosphate buffer solution KH2PO4(concentration of 0,01 Molar and pH =7), these samples distributed into Eppendorf-tubes of volume 1,5 ml. The mixture was stirred inside vortex mixer for a minute to make the compound homogeneous before exposed to low centrifugal force of 250(rpm) for half an hour at laboratory's temperature. Afterwards, the mixture had been incubate at temperature of 4°C for a whole night before exposed to centrifugal force of 10,000 Xg for 2 minutes. The supernatant was tested biological through inoculating indicator plant Nicotiana tabacum var. xanthii which topical local responded, and then Potato virus x and tobacco mosaic virus both were tested by DAS-ELISA (Double antibody sandwich - ELISA).

Soil Analysis:

Soil samples of 250 gm. adopted in this research was dried out and passed through sieve holes of 2 mm, then underwent laboratory tests to study its physical and chemical properties as shown in (Table 1) according to methods proposed by (Carter, Gregorich, 2008).
3. Results and Discussion:

Some Physical and Chemical properties of Field Soil based on table (1):

Based on table (1), has been concluded that field soil contains two textures: clay texture which is characterized by containing medium of organic matter, pH tends to acidity, and of medium level of saltiness given its capability of electrical conductivity, the second is a sandy texture characterized by low organic matter, neutral in its basal medium, and it's saline due to its capability of electrical conductivity.

| Texture | Na  | K   | Mg  | Ca  | CEC | OM   | EC  | pH 1:1 | Kg⁻¹.gm silt | Kg⁻¹.gm clay | Kh⁻¹.gm sandy |
|---------|-----|-----|-----|-----|-----|------|-----|--------|---------------|---------------|---------------|
| Clay    | 44  | 0.50| 1.4 | 11  | 31  | 20   | 5.85| 6.11   | 440           | 380           | 220           |
| Sandy   | 20  | 0.42| 0.4 | 3   | 16  | 12   | 4.65| 8.10   | 140           | 350           | 510           |

(Cation Exchange Capacity) = CEC, (Org. Mater) = OM, (Electrical Conductivity) = EC

Some studies indicated that soil of high clay content was that of high virus adsorption capacity (Gebra et al. 1975 and Bitton et al. 1978). This study has figured out that high clay content of 440 gm. Kg⁻¹ (Table 1) had been in clay soil texture and there was conformity between biological-test (virus concentration) and ELISA test of clay content in soil texture (Table 2), which means that virus adsorption lies in this texture. Clay granules in soil has impact on soil properties (Hillel, 1982) which causes increase in area unit in relation to mass unit. While (Chu et al. 2003) indicated that incorporation of viruses with the extended clay minerals of type 1:2 might have attributed to the superficial area of the clay granules as well as high efficiency of cation exchange capacity which is considered as property existent in the clay. It's been inferred that increase of exchanged cation especially the binary like Ca⁺⁺, Mg⁺⁺ (table 1), in addition to Cation exchange capacity increase in soil led to viruses’ adsorption in clay soil. (Ali and Jamal, 2013), both indicated that decrease of pH, rate of soil filtration and the ions concentration increase in the soil solution could all play a significant role in increasing the adsorption of Tobacco mosaic virus on soil granules, while, high rate of filtration and low clay content in the sandy texture soil have resulted in loss of virus in soil. Thus, viruses' adhesion to clay's minerals or metal occurs during the formation of cation bridges between capsid negative charges network and network of negative charges of clay minerals as proposed by (Carison et al. 1986). Additionally, it has demonstrated that an existence of two viruses in soil is changed during the year where their concentrations in fall yield was less than what had been in spring yield at potato farms. Such a case can be attributed to soil humidity (moistness) in fall which caused viruses migrating to lower soil layers, also humidity increase may assist in activating fungi and bacteria (considered as microflora) that act in contrast to viruses (Kegler,1993 and Sparr et al. 1995). It may also attribute to decrease of nutrients proportion including proportion of soil minerals which connected with viruses’ concentration in it (Table 2). Therefore, it's deemed as one of logical reasons which pushes farmer to add fertilizer to soil during fall season.

Accumulation of Potato virus X (PVX) and Tobacco mosaic virus (TMV) in soil:

Biological-tests through plants inoculating Indicator plants conducted on soil samples taken from potato farms revealed that soil had been infected and polluted by both PVX and TMV. High concentration of Potato virus X occurred after pollinating plants by clay soil extract compared to number of topical local lesion on tested plants when pollinated by extract of sandy texture soil. While there were no symptoms appeared on comparison control represented by inoculated leaves of plants of
tobacco by unpolluted soil extract of both textures as shown in (Table 2- Figure 2). Based on results, the existence of Tobacco mosaic virus (TMV) was detected in soil only, while Potato virus X (PVX) was existent in soil and plants which means that some of viruses can only be existent in soil and not in plants and vice versa according to the appropriateness of soil properties. (Papko *et al.* 2001) revealed that Peach dwarf virus (PDV) and Arabis mosaic virus (Ar MV) had been existent in both soil and plants, while Cucumber leaf spot virus (CLSV) had been existent in soil only.

(Figure 2) A: Tobacco leaf inoculated by clay soil extract polluted by potato virus X.
B: Tobacco leaf inoculated by sandy soil extract polluted by potato virus X.
C: Control- tobacco leaf inoculated by unpolluted soil extract.

**Table 2:** Viral concentration of Potato virus X (PVX) and Tobacco mosaic virus (TMV) in clay and sandy soils polluted by virus in terms of local lesions number on tobacco plants and ELISA absorption values.

| ELISA absorption at 405nm | DAS-ELISA test | Rate of local lesions number on N. tabacum var. xanthii | Soil texture | Virus  |
|--------------------------|----------------|--------------------------------------------------------|--------------|--------|
| 0.634                    | +              | 23                                                     | clay         | TMV    |
| 0.478                    | +              | 19                                                     |              | PVX    |
| 0.380                    | +              | 12                                                     | Sandy        | TMV    |
| 0.360                    | +              | 10                                                     |              | PVX    |
| 0.019                    | -              | 2                                                      |              | **control** |

*Rate of local lesion number on three plants inoculated in growth phase for five leaf
**control: unpolluted soil for both of sandy and clay textures*

(Kegler, 1993) indicated that there were four means methods divided into two direct and other indirect methods that could pollute soil and water by such viruses. First direct method involves virus infected derbies waste after cultivation season, which remains in roots, stems, tubers and chromates etc., and would later represent pollution source, while second direct method of soil pollution by these viruses can be indicated by infected plant roots extracts in soil and water, polluted nutritious solutions by these viruses that transmit to surroundings soil, as well as groundwater through polluted soil washing by waters of rains and flood (Kegler *et al.* 1982). These methods can be summarized that virus particles can get out through infected plant roots that would remain active in the medium surrounding these roots, this process been demonstrated by (Yarwood, 1960). Tobacco mosaic virus
(TMV), Tobacco necrosis virus(TNV) and Tomato bushy stunt virus(TBSV) are deemed examples of those viruses. However, this method hasn't yet been proven on Cucumber mosaic virus(CMV) (Buttner, 1994).

Regarding the indirect two methods can be explained as: first method involves rinsing viruses off polluted soil via groundwater or waters of rain and flood in which being transmitted later to virus free soil. Second method arises from the role of humans and grazing animals that feed on polluted plants or on parts of where these viruses pass through digestive channels without losing their ability to infection to be subtracted with animals' feces to virus free environment whether is soil or water (Tomlinson et al. 1982 and Kegler et al. 1984). viral pollution in contaminate areas is characterized by being long-last and can be lowered or put down with time but hard to be removed (Kegler et al. 1995).

According to elaboration of the biological and epidemiological characteristics mentioned previously for this group of viruses, pollution of soil and water by infected plants has become unavoidable. Consequently, precise diagnosis is considered as means condition for taken measures against these viruses that existent in soil, water and debris plant, that represent economic and environmental challenge. Moreover, a comprehensive analysis must be conducted on all weeds as being flora, that represent a source or secondary families for virus and may act as source of continuous infection.

4. References

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