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# Development of Dot-ELISA Technique for Detection of Chlorpyriphos Pesticide and Feasibility of its Application to Field Samples

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**Abstract:** ***The present study was undertaken to develop an immunoassay technique - Dot ELISA. This technique utilized nitrocellulose membrane for chlorpyriphos detection in field samples through blot appearance on it. Present study has been focused on detection of chlorpyrifos pesticide in field sprayed samples(wheat leaves, cabbage and orange) resulted in limit of detection LOD value of 75-125µg/ml through Dot ELISA. Results obtained from this study showed expected values with specific sensitivity, specificity, accuracy and K value > 0.81 using kappa statistics.These studies were further extended for comparision with Gas Chromatography (GC) and indirect plate ELISA for which LOD values were found to be of 0.1 ng/ml and 75 ng/ml with 81-94% recovery in each case respectively. So studies revealed that Dot ELISA has almost comparative value to indirect plate ELISA in concern to its LOD and % recovery. These comparative studies of all the three methods used i.e. GC, plate ELISA and Dot ELISA revealed about capability for pesticide residue detection with different level of sensitivity and detection limit. Results also supported that Dot ELISA can be consider an important tool for detection of chlorpyrifos residue even at field level which is not feasible with GC, and indirect plate ELISA.***

**Keywords:** Gas Chromatography, Indirect plate ELISA, kappa statistics, LOD, Percent Recovery

## 1. Introduction

Pesticides are used globally for enhancing crop yields. However, their excessive use/misuse, especially in the developing countries, results in widespread food and environmental contamination. Therefore current methods such as gas chromatography and high-performance liquid chromatography have been used successfully, with great sensitivity and reliability, for detection of many pesticides with minimum LOD values. However, these classical methods require a high capital expenditure and skilled analysts including time-consuming sample preparation steps. Therefore, there is a growing demand for more rapid and economical methods like immunoassay (plate ELISA and Dot ELISA) for determining pesticide residues. These methods have been emerged as an alternative to traditional methods to meet such demands of fast, sensitive and cost-effective tool for pesticide residue analysis. Since plate ELISA cannot be applied at field level because of its heavily designed equipment, so it needs to be extended to the format of dot ELISA. The dot ELISA is a qualitative ELISA test [1]whichcan be performed more quickly without the need of equipment particularly for pesticide residual detection at field samples e.g. fruit, fodder and vegetables through Dot ELISA. The certain comparable values of sensitivity, specificity, accuracy and K value > 0.81 has been obtained by using kappa statistics[2].Therefore relative speed and simplicity of dot ELISA make it an attractive alternative to standard plate ELISA. This technique can even detect at nano-gram scale among targeted compounds in situ.

## 2. Literaure Survey

Though ELISA's have been developed for the detection of various pesticides, a few attempts have been made for CPF (Cho *et al.*[3]and Brun *et al.*[4]. Even in those, the detection assays previously developed had low sensitivity limits. Moreover not all immunoassays are completely specific to one single pesticide. Although highly sensitive plate ELISA has been developed for the detection of chlopyrifos but its performance has not been compared with the most commonly used classical methods GC/HPLC. It has also not been evaluated with field samples. Further since plate ELISA cannot be applied at field level, so it needs to be extended to the format of Dot ELISA which is based on simple principle. The Dot ELISA is a qualitative ELISA test, which can be performed more quickly without the need of equipment or technical expertise has highly desirable. Dot ELISA is a micro ELISA utilizing antigen "dotted" onto nitrocellulose filter discs that had been used for more than 25 years.).

Different studies had been conducted on Dot ELISA method for the detection of various diseases like viral bacterial and parasitic. The qualitative and quantitative detection of aflatoxin B1 in poultry sera was done by ELISA (Sekhon *et al* [5]). In another supporting study Dot dye immunoassay for the diagnosis of *Schistomasis mansoni* was done (Xue *et al* [6]). Although spots obtained in Dot ELISA are slightly more intense than in dot double immunodiffusion assay (DIA), but provides the preliminary substitute with advantage of serological diagnosis of *S.mansoni.*

A multidot immunoblot assay using locally available NCM was described from 42 sera bacteriologically confirmed case of pulmonary tuberculosis. Fourty four sera from apparently healthy age and sex are taken and sensitivity of test is found to be 95% and the specificity 92%. Results showed that there is a good correlation of Dot ELISA with micropipette plate ELISA (Rattan and shriniwas [7]).


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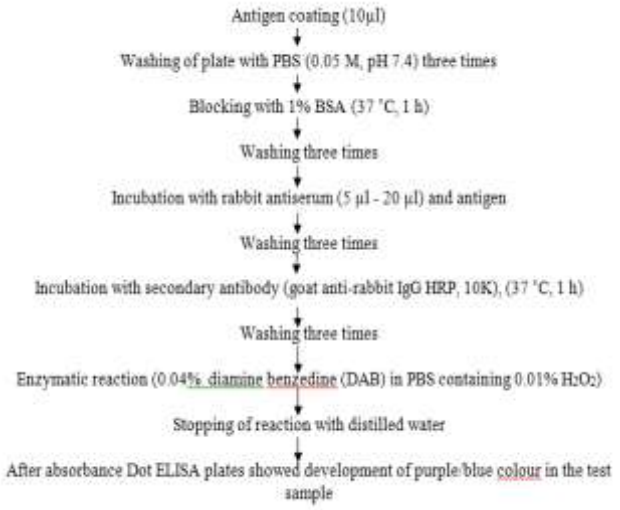
## 3. Problem Definition

The easy availability of nitrocellulose membrane (NCM) and the fact that the paper strips can be retained as permanent record for reference purpose, make this test suitable for most laboratories in the tropics. Because of its relative speed and simplicity, the Dot ELISA is an attractive alternative to standard ELISA. This technique can even detect at nano-gram scale among targeted compounds *in situ* at industry level.

## 4. Methodology/ Approach

### 4.1. Design of immunocomb for Dot ELISA

Nitrocellulose membrane (NCM) strips of 5 x 5 $mm^2$ wasmarked with lead pencil at 1 cm intervals for orientation of antigen Dots. After that (NCP) were coated separately with 2 to 3µl of field sample for the test and CPF spiked samples as (positive control). The negative controls coated with preparation from CPF water free samples. The coated NCM strips were dried at 65°C for 2 h in an incubator and then blocked in PBS containing 0.05% Tween -20 (PBS-T). Then plate was incubated with 2 to 3µl of prepared antigen with the above concentrate .Thereafter presence of CPF pesticide in the field samples were qualitatively detected by following method:




**Flow chart 1:** Protocol for development of Dot ELISA for the detection of chlorpyrifos.

### 4.2. Standardization of Dot ELISA for detection of chlorpyrifos pesticide at laboratory level

Development For standardization anddevelopment of Dot ELISA first of all limit of detection (LOD) forpurechlorpyrifos and prepared antigen were calculated by following the procedure as given in Flow chart 1. After obtaining the cut of dose for chlorpyrifos detection free water samples, as detected by GC and indirect plate ELISA[4]. The samples were spiked with different concentrations (1000-0.1µg/ml) of pure chlorpyrifos. An un-spiked/negative control was also maintained for comparison. The limit of detection (LOD) described in Table.1,was calculated for Dot ELISA method [2].

### 4.3. Development of Dot ELISA for detection of chlorpyrifos in field samples

For Dot ELISA antigen concentration were used: 1000, 500,250, 125,75, and 50µg/ml with antibody dilution (1:1000 µl/ml) and 1:1500 HRPO conjugate dilution. Sensitivity of this methods was compared with GC and indirect olate ELISA by calculating their limit of detection (LOD Higher the LOD value was directly proportional to the sensitivity of the test/method.

### 4.4. Collectionof field samples

Different samples of agricultural produce were collected from three separate local markets of Ludhiana i.e Clock tower, Ghumar mandi and Agar nagar. Samples were divided into three groups: I, II and III i.e. fruits, vegetables and fodder respectively. All the samples were analyzed for the detection of chlorpyrifos residue by three methods: gas chromatography, indirect plate ELISA and Dot ELISA.

### 4.5. Preparation of field samples for development of Dot ELISA

All the samples of three groups i.e. I, II and III were weighed to 50g and was homogenized by using 100 ml of methanol with high speed homogenizer for 2 minutes. Residue was concentrated by removing excess of solvent and collected 10 ml of residue it by using rotary evaporator. From concentrate of 10 ml, 1ml of residue was taken and made the volume of 10 ml with PBS (pH 7.6) i.e. in the ratio of 1:9. Now samples were ready to utilize for coating of as antigen on Dot ELISA comb.

### 4.6. Statistical analysis for Dot ELISA

The sensitivity, specificity and accuracy of Dot ELISA was compared with indirect plate ELISA by (neutralization test) agreement between antigen and antibody as described[2].
Where, Sensitivity: a / (a+c)
Specificity: d / (b+d)
Accuracy: a+d / (a+b+c+d)
K = (a+d – P) / 1- P,
Where P= (a+b) (a+c) + (c+d) (b+d) and P is the probability,
a: is the number of samples positive by both i.e., test to be compared and gold standard test.
b: is the number of samples positive by standard test whereas negative by test to be compared.
c: is the number of samples negative by standard test and positive by test to be compared.
d: is the number of samples negative by both.
k value > 0.81 indicates perfect agreement.

## 5. Result and Discussion

### 5.1. Detection limit for synthesized and pure CPF , anti chlorpyrifos antibodies (ACAb) through Dot ELISA technique

After determining the sensitivity limits and LOD for ACAb by two methods in the previous sections i.e. indirect plate ELISA and GC[8].The study was further extended to standardize and to develop Dot ELISA method for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qualitative detection of CPF pesticide in the spiked samples as well as in the field samples following the method given (flow chart 1). Results obtained are discussed below:

**5.2. Standardization of Dot ELISA for detection of anti chlorpyrifos antibodies**

Two types of CPF antigens i.e. pure and synthesized were used forstandardization of Dot ELISA technique to detect anti CPF antibodies (ACAb). The cut of dose i.e. LOD for CPF obtained from theperformance of indirect plate ELISA was 100 ng/ml, with 1:100 of antibody dilution above that dose no colour was developed therefore it showed 100% binding inhibition. Thus this value of LOD i.e. 100ng/ml of CPF was also considered for conducting of Dot ELISA experiment. But as this dose did not work for development of colour, therefore dose was further enhanced so as to determine the LOD of antigen required for Dot ELISA.

In case of pure CPF results showed the agreement (colour development) at the dilution range of antigen and antibody i.e 1µg/ml and 1:1000, respectively which has been represented by positive sign in table 1, with its corresponding visual colour development as shown in Plate-I, Fig. B. While in case of prepared CPF antigen, it showed agreement at the dilution level of (100µg/ml and 1:1000 µg/ml) which has been represented by the positive sign in table 1, corresponding to colour development in plate- I, Fig B. Therefore, results showed higher LOD for pure CPF as compared to that of prepared CPF antigen. This interpretation of results supported that the commercially available CPF can be detected by Dot ELISA in the field samples, even if these samples contained certain amount of pesticide with in the threshold LOD obtained by Dot ELISA i.e. 1 µg/ml. The results in present study also implied that even though Dot ELISA has LOD of 1µg/ml CPF, which was found to be lower as compared to the previous diagnostic test used for CPF pesticide i.e. indirect plate ELISA, where its value was 100ng/ml and competitive inhibition ELISA with its value 10ng/ml, but still it is sufficient for rapid and accurate detection of CPF pesticide with certain LOD [8]. Earlier studies for the organophosphorus detection in water samples showed the LOD of 1.4 and 1.2µg/ml by Dot ELISA and plate ELISA[9].This study supported the results obtained in present study. Literature also revealed that Dot ELISA has been used for the diagnosis of infectious bursal disease in chicken and found LOD equal to 2.1 µg/ml with antibody dilution of 1:500 described [10].In another study standardized of Dot ELISA for serodiagnosis of tuberculosis on nitrocellulose membrane[7] and found LOD equal to 1.8 µg/ml with antibody dilution of 1:400.

**5.3. Detection of chlorpyrifos spiked water samples by DOT ELISA**

Chlorpyrifos free samples spiked with different concentrations of pure CPF were analysed for its detection by following the technique of Dot ELISA for comparison of results obtained from indirect plate ELISA. Different spiked concentrations of pure CPF in duplicates showed that Dot ELISA was found to have LOD with value of 100 µg/ml for CPF with 1:100 of antibody dilution i.e. above this range no colour development was observed (Table 2). While the results obtained with indirect plate ELISA for spiked water samples showed LOD for CPF 100 ng/ml of antigen with 1:1000 of antibody dilution (Table 2). Therefore, the comparative studies of LOD between Dot ELISA and indirect plate ELISA interpreted that even though Dot ELISA has lower LOD as compared to indirect plate ELISA, but it is able to detect CPF at the level of certain amount therefore, it can be adopted as a tool for pesticide detection.

**5.4. Detection of chlorpyrifos spiked field samples by Dot ELISA**

Homogenates prepared separately from all the field samples (three replicates each) were detected through Dot ELISA (Table.3) by using only two dilution of antigen i.e spiked field sample (1000 and 100 µg/ml for Dot ELISAand 1000 and 100 ng/ml for indirect plate ELISA) with single antibody dilution (1:1000 µl/ml) and 1:1500 HRPO dilution**.** The results obtained from the tests (indirect plate ELISA and Dot ELISA) were analyzed for the percentage of agreement between antibodies and antigen (field samples).The field spray samples were also detected for the presence of CPF by Dot ELISA using different concentrations of pure CPF (1000, 500, 250, 125, 75 and 50 µg/ml) were sprayed on wheat leaves, cabbage and orange which were detected by Dot ELISA using antibody with 1:100 dilution. Qualitative analysis indicated LOD for CPF as75 µg/ml for wheat leaves, 125µg/ml for cabbage and 75 µg/ml for orange samples as above this LOD value no colour development was observed (Table 3). The study was undertaken to standardize and evaluate Dot ELISA for the direct detection of A. hydrophila from diseased fish samples and to confirm suspected A. hydrophila cultures isolated from the samples. In another case study Dot ELISA wasalsodeveloped for detection of antibodies to infectious bursal disease, hydro pericardium syndrome (HPS) and chicken anemia viruses (CAV) [11]and this test was found to be highly sensitive, specific with a K value of 0.99 even at the level of 102 µg/ml of infection.by using Kappa statistics[2]as described in section 3.16.2 and To find out the efficacy of both the methods i.e. Dot ELISA and indirect plate ELISA, the LOD found from Dot ELISA test was taken into consideration for comparison.

**5.5. Comparison for LOD of Dot ELISA and plate ELISA for chlorpyrifos detection in field sprayed samples**

To find out the efficacy of both the methods i.e Dot ELISA and indirect plate ELISA, the LOD found from both the tests were taken into consideration for comparisons at the same level (Table 4). In case of wheat leaves it was observed that out of 20 samples, 9 were found to be positive by Dot ELISA and 14 were positive by indirect plate ELISA, while 6 negative both by Dot ELISA and 6 also negative byindirect plate ELISA at 75µg/ml (Table 4). In case of cabbage, it was found that out of 18 samples, 9 found to be positive by Dot ELISA and 12 were positive by indirect plate ELISA while 8 negative by Dot ELISA and 6 by indirect plate ELISA at 125µg/ml. So,it was observed that out of 20 samples of orange 10 were found to be positive by Dot ELISA and 15 were positive by indirect plate ELISA,


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while 10 negative by Dot ELISA and 5 by indirect plate ELISA at 75µg/ml (Table 4).

During the study four other parameters viz. sensitivity, specificity, accuracy, and K value were also determined as shown in tables 4.These results showed K value greater than 0.81 in all the field samples indicating perfect agreement between two techniques followed i.e. plate ELISA and Dot ELISA. This showed that both of the techniques were comparable for detection of pesticide residue detection at 75-125µg/ml dilution of antigen for Dot ELISA and 75-125ng/ml dilution of antigen among the field sprayed samples. Literature reveals that most of the work in the field Dot ELISA has been carried out for the qualitative detection of various diseases and reports on such study regarding the pesticide residue analysis in the field samples is meager. Therefore the present results have been justified on the basis of work done in clinical aspects. Literature also revealed that the overall sensitivity of the Dot ELISA test was found to be 78.9% with tissue infected with *A. hydrophila in* fish, while it showed 92.8% sensitivity with direct plate ELISA in field conditions [12]. Development of a Dot-ELISA assay for diagnosis of southern rice black-streaked dwarf disease (SRBSDV) in the field of suspected rice was found to be positive for SRBSDV by the Dot-ELISA and confirmed by the One Step RT-PCR method[13]. A multidot immunoblot assay was performed for diagnosis of pulmonary tuberculosis by using locally available nitrocellulose membrane (NCM) and it was found that 42 sera were bacteriologically infected with pulmonary tuberculosis with sensitivity of 95% and the specificity of 92 %, thus indicating a good correlation of Dot ELISA with micropipette plate ELISA [7].

Various viral, bacterial and parasitic diseases have also been detected by Dot ELISA assay and the finding of these studies has shown effective applicability of this technique [14-16]. The results obtained in the present study can also be supported by the facts observed from another study [17]which reported ELISA as a sensitive, specific, effective and suitable immunoassay for detection of chlorpyrifos residue in field samples as compared to other conventionally used techniques.

## 6. Conclusion

These results interpreted that all the three methods used i.e. GC, plate ELISA and Dot ELISA were equally sufficient and capable for pesticide residue detection with different level of sensitivity and detection limit. Results also supported the feasibility of Dot ELISA for pesticide residue detection even at field level with LOD range of 75-125µg/ml and hence would be very useful for the detection of chlorpyrifos residue in the field samples.

## 7. Future Scope

Dot- ELISA results were compared with conventional tests and indiect plate ELISA. When dot- ELISA was performed there was negative reaction for these isolates. To cross check the indiect plate ELISA test was carried out as mentioned above the dot-ELISA and indiect plate ELISA results correlated with each other therefore, Dot- ELISA can be adopted for detection and it is also proven to be cost effective.

## References

[1] Parmar P, & kocher D K, Study of Immunological Parameters from the Serum of Rabbits Exposed to Chlorpyrifos Pesticide Research. *Indian journal of app research*, 2013, pp.15-17.
[2] Venkatesh G, Development of recombinant antigens for diagnosis of avian mycoplasmosis, Ph.D. thesis,Tamil Nadu Veterinary and Animal Sciences University, Chennai, 2006.
[3] Cho Y A, Lee V, Park E Y, Lee Y T, Bruce D H, Chang A K and Jae K LDevelopment of an ELISA for the Organophosphorus Insecticide CPF. *Bull Korean Chem Soc*, 2002, pp**.** 481-87.
[4] Brun, A, Castilla, J, Ramirez M A, Prager K, Parra B, Salguero F J, Shiveral D, Sanchez C, and Sanchez-Vizcaino J M (Proteinase K enhanced immunoreactivity of theprion protein-specific monoclonal antibody 2A11. *Neurosci Res***.**2005, pp. 233-34.
[5] Sekhon C P S, Kapur J, & Sodhi S S, Production of Anti-aflatoxin B1, antibodies in rabbits and development of an immunoassay for their detection. Annual biology.1996, pp. 15-17.
[6] Xue H C, Quiu L S, Zang Y H and Zhu Z X (Dot ELISA in the diagnosis of schistomiasis.*Clin Med* .1987 pp. 113-15.
[7] Rattan A, & Shriniwas B, Standardisation of nitrocellulose membrane Dot ELISA for serodiagnosis of tuberculosis a technique suitable for india. *Indian journal of tuberculosis.*1990, pp. 17-23.
[8] Parmar P, & kocher D K, Comparative study of immunoassay and Gas chromatography for chlorpyrifos detection in laboratory spiked water samples. International journal of scientific research. 2017, pp. 678-680.
[9] Gordon J, & Biling P, Dot immunobinding,General principle and procedure. Hand book of immunoblotting of protein Ed. Bjerrum oj.,Hecgeard N.H.H.VolCRC, Inc., Boca Raton. 27 (1988) (in press).
[10] Sultana J, Hussain M, Malik S, & Naeem K, Use of dot ELISA for the diagnosis of infectious bursal disease in chicken.Pakistan Vet.1999, pp. 139-142.
[11] Subramanyam V, PurushothamanV, MuraliManohar G G, kumar R, & Manoharan S, Development of Dot – Enzyme Linked Immunosorbent Assay for Detection of Antibodies to Infectious Bursal Disease, Hydropericardium Syndrome and Chicken Anaemia Viruses. European journal of Biological Science, 2010, pp. 08-11.
[12] Shome B, & Gaur K K, Rapid detection of Aeromonas hydrophila infection in freshwater fish by Dot ELISA. Indian journal of fishes. 2005, pp.269-272.
[13] Wang K, Zheng J J S, Zhang G, & Zhou H, Seedling test confirms Southern rice black-streaked dwarf virus cannot be transmitted via rice seed. Guangdong Agriculture science.7 2010, pp. 95-100.
[14] Romer R T, Screening methods for the detection of aflatoxins in mixed feeds and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxins in positive


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samples. Association of Official Analytical Chemists.1975, pp.500-503

[15] Cocker R D, Jones B D, Nagler M R, Gilman G A Wallbridge A J, & Panigrahi S, Tropical Development and Research Institute (1984) Mycotoxin Training (TDRI) Manual London.

[16] Young J G, Eskenazi B, Gladstone E A, Bradman A, Pedersen L, Johnson C, Barr D B, Furlong C E, & Holland N T, Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. NeuroToxicology.2005, pp. 199-202.

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**Table 1:** Detection of antichlorpyrifos antibodies with synthesized antigen and pure chlorpyrifos through Dot ELISA

| Type of antigen | Positive control | Antigen dilution µg /ml | | | | | | | Negative Control |
|---|---|---|---|---|---|---|---|---|---|
| | | 1000 | 100 | 10 | 1 | 0.1 | 0.01 | 0.001 | |
| Pure CPF | + | + | + | + | + | - | - | - | - |
| | + | + | + | + | + | - | - | - | - |
| | + | + | + | + | + | - | - | - | - |
| Synthesized antigen | + | + | + | - | - | - | - | - | - |
| | + | + | + | - | - | - | - | - | - |
| | + | + | + | - | - | - | - | - | - |

+ sign represents colour development
-sign represents no colour ,Nil
Antibody dilution 1:1000 in all

**Table 2:** Detection of spiked concentrations of pure chlorpyrifos in water samples through Dot ELISA

| Type of antigen (CPF) | Positive control | Antigen dilution µg /ml (comb 1-7) | | | | | Negative control |
|---|---|---|---|---|---|---|---|
| | | 1000 | 100 | 10 | 1 | 0.1 | |
| Replicate-1 | + | + | + | - | - | - | - |
| | + | + | + | - | - | - | - |
| Replicate-2 | + | + | + | - | - | - | - |
| | + | + | + | - | - | - | - |

+ sign represents colour development
-sign represents no colour
Antibody dilution 1:1000 in all

*Table 3. Dot ELISA for detection of chlorpyrifos residue in field sprayed samples*

| *Sample tested* | *Positive control* | *Antigen concentration (µg/ml)* | | | | | | *Negative control* |
|---|---|---|---|---|---|---|---|---|
| | | 1000 | 500 | 250 | 125 | 75 | 50 | |
| Wheat leaves | + | + | + | + | + | + | - | - |
| | + | + | + | + | + | + | - | - |
| | + | + | + | + | + | + | - | - |
| Cabbage | | 1000 | 500 | 250 | 125 | 75 | | |
| | | + | + | + | + | - | | |
| | | + | + | + | + | - | | |
| | | + | + | + | + | - | | |
| Orange | Positive control | 1000 | 500 | 250 | 125 | 75 | 50 | Negative control |
| | + | + | + | + | + | + | - | - |
| | + | + | + | + | + | + | - | - |
| | + | + | + | + | + | + | - | - |
| Antibody dilution 1:100 in all | | | | | | | | |

**Table 4:** Detection of chlorpyrifos through Dot ELISA and indirect plate ELISA in sprayed field samples

| *Sample Tested- Wheat leaves* | | *Indirect plate ELISA- 75 µg/ml* | | |
|---|---|---|---|---|
| | | *Positive* | *Negative* | *Total* |
| Dot ELISA-75 µg/ml | Positive | a=6 | b=3 | 9 |
| | Negative | c=8 | d=3 | 11 |
| | Total | 14 | 6 | 20 |
| Sensitivity: 82%, Specificity: 85% Accuracy: 86%, K-value: 0.97 | | | | |


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| Sample Tested- Cabbage | | Indirect plate ELISA- 125 µg/ml | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Dot ELISA-125 µg/ml | Positive | a=7 | b=3 | 10 |
| | Negative | c=5 | d=3 | 8 |
| | Total | 12 | 6 | 18 |
| Sensitivity: 86%,<br>Specificity: 84.3%<br>Accuracy: 88%,<br>K-value: 0.99 | | | | |
| Sample Tested- Orange | | Indirect plate ELISA- 75 µg/ml | | |
| | | Positive | Negative | Total |
| Dot ELISA-75 µg/ml | Positive | a=8 | b=2 | 10 |
| | Negative | c=7 | d=3 | 10 |
| | Total | 15 | 5 | 20 |
| Sensitivity: 83%,<br>Specificity: 85.2%<br>Accuracy: 87%,<br>K-value: 0.98 | | | | |

Plate.1, Fig. A

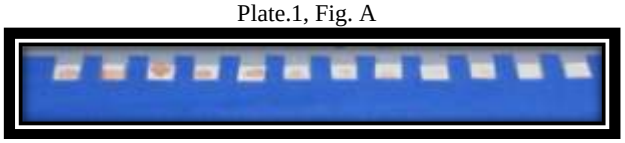

Plate 1, Fig .B

**Plate 1:** Fig. A Pure chlorpyrifos and Fig .B Synthesized chlorpyrifos hapte


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