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RESEARCH ARTICLE

# In vitro Evaluation of Insecticides, Bio-Fungicide and Bio-Fertilizer for Strategic and Eco-Friendly Combinatorial Seed Treatments in Chickpea

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**Abstract** Use of seed treatments in Integrated Pest Management of major field crops has increased considerably over the past few years. However, field recommendations are seldom supported by in vitro assays screening the suitability of the pesticides for seed treatment. Hence, the present investigation was undertaken to assess the suitability of three insecticides as imidacloprid (600 FS), fipronil (5 FS) and chlorpyriphos (20 EC) for seed treatment in chick pea as well as to verify the compatibility of identified doses of test-insecticides, along with biofertilizer (*Mesorhizobium ciceri* @ 25 g/kg seed) and in combination with biofungicide (*Trichoderma harzianum* @ 4 g/kg seed). Ten treatments were evaluated in insecticidal studies viz. control and three treatments of each insecticides i.e. nine treatments) and eight treatments were evaluated in compatibility study. Based on the results obtained, the authors advocate appropriate IPM-compatible and eco-friendly seed treatment tactics, viz., (1) imidacloprid @ 5 ml/kg + *Mesorhizobium*, (2) imidacloprid @ 5 ml/kg + *Mesorhizobium* @ 25 g/kg seed + *Trichoderma* @ 4 g/kg seed, (3) fipronil @ 5 ml/kg + *Mesorhizobium* @ 25 g/kg seed, and (4) fipronil @ 5 ml/kg + *Mesorhizobium* @ 25 g/kg seed + *Trichoderma* @ 4 g/kg seed. These recommendations may be tested for the field level efficacy and the insecticides tested hereby are essentially termiticides, but recommendations can well be adopted for sucking insect pests for good agricultural practices in chickpea.

**Keywords** Chlorpyriphos · Fipronil · Imidacloprid · *Mesorhizobium* · *Trichoderma* · Seed treatment

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## Introduction

Chickpea is the third most important pulse crop, after dry bean and peas, produced in the world. It accounts for 20 % of the world pulse production. Major producers of chickpea include India, Pakistan and Mexico. Global production, as per the latest available estimates of Food and Agricultural Organization (FAO) is about 12 million metric tons in 2011. India is the largest producer, with about 8 million tons, accounting for about 70 % of total world production. Six countries including India, Australia, Turkey, Myanmar, Pakistan and Ethiopia account for about 90 % of world chickpea production [1]. Chickpea being considered as drought tolerant crop, is grown under rainfed conditions, which survives the progressive drought conditions from its planting in post-rainy season to harvest [2, 3]. Such crop growth conditions are quite congenial to termite infestation in tropical countries like India. In areas like Rajasthan and Haryana, where termite infestation is more [4], Chickpea is ravaged by various sucking insect-pests prevalent in the early crop stage [5] in addition to termites, which could be addressed by seed treatment.

Seed treatment plays a crucial role in protecting the emerging seedlings from insect-pests and diseases which deal under its purview, both products and processes. The usage of specific products and specific techniques can improve the growth environment for the seeds, seedlings

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and young plants. Today, seed treatment has evolved into a more complex science. An ideal chemical seed treatment should be highly effective against various pests and diseases, simultaneously satisfying other requirements which should be harmless to the seed and seedlings to offer an even coating to seed, adhere well, relatively inexpensive and of course are registered for the intended use. The pesticides used in seed treatments should not affect adversely the seedling or plant growth and development. Furthermore, insecticide seed treatments are often formulated as combinations with other seed protectant pesticides, biofertilizers and biocontrol agents. In Good Agricultural Practices (GAP), inclusion of biofertilizer and biofungicide in the seed treatment also adds another dimension for a holistic approach. Seed treatment is an eco-friendly disease and pest management tactic that is gaining much importance as an IPM-compatible measure. Use of seed treatments on cereals, pulses and oilseed crops has increased considerably over the past few years worldwide including India. Recommendation of seed treatment for various field crops is offered by Government of India under its most ambitious programme *Total Seed Treatment Campaign* since 2007 [6]. Seed treatments protect seed from insect pests such as the jassids, aphids, shoot fly and termites and diseases like wilt and rot.

Insecticides like imidacloprid, fipronil and chlorpyriphos are proven to be effective against termite control and a valid IPM-option for termite control in India. Soil application of these termiticides may be disadvantageous due to their percolation deep into soil, may not be in the vicinity of plant roots at right time, huge requirements and may not be eco-friendly. To address these problems, application of termiticides as seed treatment could be used as one of the alternatives. IPM packages of Govt of India, Ministry of Agriculture recommend for chickpea seed treatment with biocontrol agents like *Trichoderma viridae* or *T. harzianum* @ 4 g/kg for diseases; and chlorpyriphos 20 EC @ 15–20 ml/kg of seed for termites. Biofertiliser like *Rhizobium* culture is also advocated. But these recommendations are in isolation, and nothing is suggested regarding combination of these pesticides as a real research gap which is true for most of the other crops as well. Hence, the present investigation was undertaken to study the effect of termiticidal seed treatments either alone or in combination with biofertilizers and biocontrol agents on chickpea seed quality attributes and to pin point the right dosage of termitecides to be used for treating the seeds either alone or in combinatorial approach of termiticide, biofertiliser and biocontrol agents.

## Material and Methods

### Test-Pesticides/Seed Protectants/Bio-Fertilizers

The details of insecticides, bio-agents, *Rhizobium* and their source of supply are given below

| Compound name | Trade name | Source of supply |
|---|---|---|
| Chlorpyriphos 20 % EC | Dursban® | Dow Agroscience of India Pvt. Ltd. Mumbai-400 071 |
| Imidacloprid 48 % FS | Gaucho® 600FS | Bayer CropScience Ltd. Mumbai-400 076 |
| Fipronil 5 % SC | Regent® | -do- |
| *Trichoderma harzianum* | Lab culture | Division of Plant Pathology IARI, New Delhi |
| *Mesorhizobium ciceri* | Lab culture | Division of Microbiology IARI, New Delhi |

### Test-Crop and Variety

Chickpea (variety, Pusa-1003) is kabuli type of chickpea with a yield potential of 2.0 t/ha. Important characteristics of this variety are tolerance to wilt and bold seeds. Seeds of test-crop were obtained from Seed Unit, Division of Genetics, Indian Agricultural Research Institute, New Delhi.

The seeds are treated with imidacloprid 48 % FS (Gaucho® 600 FS), fipronil 5 SC (Regent®) and chlorpyriphos 20 EC (Dursban®) each at three different doses (Table 1). An untreated control (treated with distilled water) was also included. Calculated doses of respective insecticides were first added to water so as to make the final required volume of 50 ml, which was sufficient to treat 1 kg of chickpea seed. The seeds were first spread on plastic trays, desired quantity of the insecticide emulsion was sprinkled on the seeds which were thereafter turned regularly ensuring uniform seed-coating. Treated seeds were then dried for 24 h under the lab conditions and subjected to standard germination test. Similar treatment was given to control seeds but only with distilled water. For combinatorial seed treatment with termiticide, biocontrol agent and biofertilizer, seeds were treated as mentioned above in a sequential manner i.e. first insecticide, second biofungicide (*T. harzianum*) and third biofertilizer (*Mesorhizobium*).

### Germination Test

Treated seeds as well as control were subjected to germination test following the between paper method [7]. Three replicates of 100 seeds from each treatment as well as

**Table 1** Germination percentage, root length, shoot length and vigour index-II for various seed-treatments (insecticides/termiticides) in chickpea variety 'Pusa-1003'

| Treatments details | Germination (%) | Root length (cm) | Shoot length (cm) | Vigour index-II |
|---|---|---|---|---|
| $T_1$: Imidacloprid (5 ml/kg seed) | $95.57_a$ [77.86] | $18.13_{ab}$ (4.37) | $12.68_a$ (3.7) | $39.18_c$ (6.34) |
| $T_2$: Imidacloprid (7 ml/kg seed) | $96_a$ [78.45] | $18.33_{ab}$ (4.4) | $12.08_a$ (3.62) | $50.56_b$ (7.18) |
| $T_3$: Imidacloprid (10 ml/kg seed) | $94.8_b$ [76.85] | $18.5_{ab}$ (4.77) | $12.3_a$ (3.65) | $59.75_a$ (7.79) |
| $T_4$: Fipronil (5 ml/kg seed) | $92.6_c$ [74.34] | $20.76_a$ (4.65) | $12.02_a$ (3.61) | $29.98_e$ (5.57) |
| $T_5$: Fipronil (7 ml/kg seed) | $86.76_c$ [68.65] | $18.88_{ab}$ (4.46) | $12.65_a$ (3.69) | $26.69_f$ (5.26) |
| $T_6$: Fipronil (10 ml/kg seed) | $84.5_e$ [66.80] | $17.5_b$ (4.29) | $9.95_b$ (3.31) | $25.91_g$ (5.19) |
| $T_7$: Chlorpyriphos (7 ml/kg seed) | $85.6_d$ [67.33] | $17.38_b$ (4.29) | $10.08_b$ (3.33) | $24.69_h$ (5.07) |
| $T_8$: Chlorpyriphos (10 ml/kg seed) | $72.3_f$ [58.26] | $16.46_b$ (4.18) | $6.65_b$ (2.76) | $10.12_i$ (3.34) |
| $T_9$: Chlorpyriphos (12 ml/kg seed) | $65_g$ [53.75] | $14.25_c$ (3.90) | $5.40_c$ (2.53) | $6.94_j$ (2.82) |
| $T_{10}$: Control (Water) | $96.5_a$ [79.31] | $17.1_b$ (4.25) | $10.51_b$ (3.39) | $30.56_d$ (5.62) |
| SE(d) | 0.858 | 0.15 | 0.09 | 0.025 |
| CD ($P = 0.05$) | [1.80] | (0.31) | (0.19) | (0.05) |

Figures in the same column followed by same subscript letters are not significantly different (P = 0.05)

Figures in parentheses [ ] and ( ) are 'arcsine' and 'square-root' transformed values, respectively. Figures are mean of 3 replicates (each replication = 100 seeds)

control were placed between two moist germination papers and rolled. Thus rolled papers were incubated at 20 ± 1 °C in a germination room for eight days. On eighth day, normal seedlings, abnormal seedlings, dead seeds, hard seeds and fresh ungerminated seeds were counted. Germination was estimated as percentage of normal germinated seeds to the total number of seeds used in the test.

### Vigour Index (VI)

In simultaneous to seed germination test, the vigour indices were evaluated according to the protocol proposed by Abdul Baki and Anderson [8]. Post germination test (eighth day), 10 normal seedlings from both treatments and each replication were selected at random and their average seedling length (plumule tip to root tip) was recorded. Similarly, ten randomly selected 10 normal seedlings were oven dried overnight at 90 °C and their dry weights were recorded. The vigour indices were calculated using the formula as mentioned below:

$$\text{Vigour Index-I} = \text{Germination } (\%) \times \text{Average seedling length of 10 seedlings (cm)}$$

$$\text{Vigour Index-II} = \text{Germination } (\%) \times \text{Dry weight of 10 seedlings (mg)}$$

### Rapid Tetrazolium Test (TZ) for Seed Viability

Ungerminated chickpea seeds were selected at the end of in vitro germination test, and conditioned by water soaking. Seed-coats were removed carefully with forceps. Uncoated-seeds were transferred to flask containing TZ solution (2, 3, 5 triphenyl tetrazolium chloride salt) and the flask was then kept at 30 °C for 24-h under darkness. Finally staining pattern was observed and on the basis of position and size of necrotic areas in the embryonic axis and cotyledons, seeds were classified as viable and non-viable (dead seeds).

### Pot Trial for Root Nodulation

This is conducted to crosscheck the effect of combinatorial treatments on chickpea root nodulation. Same eight treatments were taken as detailed in Table 2. Five seedlings in each replication were raised in the pots after required seed-treatments in desired sequence essentially. The pots were maintained inside the walk-in-room germinator (temp. 20 °C, relative humidity 70–75 %) as per the International Seed Testing Association [8]. Chickpea seedlings were watered at 6-days interval. Final observations on root nodules were taken 30-days after sowing.

### In vitro Compatibility Test for Insecticides and Biofungicide

Study was carried out to evaluate the compatibility of test insecticides i.e. imidachloprid, fipronil and chlorpyriphos against most effective biocontrol agent (biofungicide) *T. harzianum*. Poison food technique described by Mesta et al. [9] was used to evaluate the deleterious effect on *T. harzianum.* Three doses of each insecticide with increasing concentrations (imidachloprid @ 5, 7 and 10 ml/kg seed; fipronil @ 5, 7 and

**Table 2** Germination percentage, seedling length and vigour indices for various seed-treatments (in combination: insecticide, bio-fungicide and bio-fertilizer) in chickpea var. 'PUSA-1003'

| Treatments details | Germination (%) | Root length (cm) | Shoot length (cm) | Total length (cm) | Vigour Index-I | Vigour Index-II | *Root nodules (no./plant) |
|---|---|---|---|---|---|---|---|
| $T_1$: im + rhizo | $97.39_a$ [80.68] | $21.21_{abc}$ (4.71) | $18.13_a$ (4.37) | $39.9_{ab}$ (6.4) | $3885.73_a$ (62.34) | $78.20_a$ (8.9) | $12_{ab}$ (3.60) |
| $T_2$: fip + rhizo | $91.67_c$ [73.24] | $20.03_c$ (4.58) | $15.63_{bc}$ (4.08) | $36.67_c$ (6.14) | $3360.58_d$ (57.97) | $69.24_d$ (8.38) | $10.33_b$ (3.37) |
| $T_3$: chl + rhizo | $85.10_d$ [67.28] | $16.66_d$ (4.20) | $13.33_d$ (3.79) | $30.33_d$ (5.6) | $2581.33_e$ (50.62) | $55.31_e$ (7.50) | $8.66_c$ (3.11) |
| $T_4$: im + tri + rhizo | $96.03_b$ [78.49] | $21.81_{ab}$ (4.78) | $18.76_a$ (4.45) | $40.93_a$ (6.48) | $3930.99_a$ (62.71) | $78.74_a$ (8.93) | $13_a$ (3.74) |
| $T_5$: fip + tri + rhizo | $93.11_c$ [74.77] | $20.62_{bc}$ (4.65) | $15.33_c$ (4.04) | $35.97_c$ (6.08) | $3349.36_d$ (57.88) | $68.90_d$ (8.36) | $11_b$ (3.46) |
| $T_6$: chl + tri + rhi | $83.33_d$ [65.90] | $17_d$ (4.24) | $13.5_d$ (3.81) | $30.83_d$ (5.64) | $2569.67_e$ (50.70) | $54.72_e$ (7.47) | $7.33_d$ (2.89) |
| $T_7$: rhizo | $95.60_b$ [67.33] | $22.47_a$ (4.84) | $16.16_b$ (4.21) | $38.5_b$ (6.29) | $3680.55_b$ (60.67) | $76.16_b$ (8.75) | $11_b$(3.46) |
| $T_8$: Control | $96.44_{ab}$ [79.14] | $20.83_{abc}$ (4.67) | $15.26_c$ (4.03) | $36.4_c$ (6.12) | $3510.31_c$ (59.26) | $70.95_c$ (7.67) | $7.66_{cd}$ (2.94) |
| SE(d) | 0.887 | 0.08 | 0.042 | 0.056 | 0.512 | 0.032 | 0.109 |
| CD ($P = 0.05$) | [1.9] | (0.17) | (0.09) | (0.12) | (1.1) | (0.07) | (0.23) |

Figures in the same column followed by same subscript letters are not significantly different (P = 0.05)

Figures in parentheses [ ] and ( ) are 'arcsine' and 'square-root' transformed values, respectively. Figures are mean of 3 replicates (each replication = 100 seeds)

* Root nodules were observed in a separate pot culture trial (after 30 days of sowing)

*im* imidacloprid 48 % FS (@ 5 ml/kg), *fip* Fipronil 5 FS (@ 5 ml/kg), *chl* chlorpyriphos 20 EC (@ 7 ml/kg), *tri* T. harzianum (@ 4 g/kg), and *rhizo* Mesorhizobium (@ 25 g/kg seed)

10 ml/kg seed and chlorpyriphos @ 7, 10 and 12 ml/kg seed) were used. Required quantity of insecticide-amended 15 ml PDA was poured into 90 mm diameter petridish. Each plate including the control was inoculated with 5-days old *T. harzianum* culture as 4 mm slug by using cork borer. The inoculated plates were incubated at room temperature of 30 ± 3 °C and the experiment was replicated four times. Colony morphology and mycelial growth were observed daily and mean colony inhibition percentage was calculated using the following formula.

$$\text{Mean colony inhibition (in \%)} = \frac{C - T}{C} \times 100$$

where C = Growth in control and T = growth in treatment.

### Combinatorial Test for Compatibility

While checking the compatibility of seed protectants, 300 chickpea seeds were taken and treated in the sequence (insecticide—biofungicide—biofertilizer) with the defined test-doses of test-insecticide and field recommended doses of bio-fungicide (*T. harzianum,* 4 g/kg) and bio-fertilizer (*Mesorhizobium,* 25 g/kg). Final observations on various seedling parameters were taken 7 days after sowing.

### Statistical Analyses

The data were subjected to one factorial ANOVA following standard statistical package (OPSTAT online, www.hau.ernet.in/opstat.html). In vitro compatibility (biofungicide and insecticides) experiment was conducted in complete randomized design (CRD). The obtained data were statistically analyzed using SAS package and the significance among the treatments was determined according to the least significant difference (LSD) at 95 % of confidence interval.

## Results and Discussion

### Insecticidal Screening

In first step of the present study, where three different doses of each insecticide have been evaluated on different quality parameters of seedling; imidacloprid treated seeds showed higher seed quality parameters, which could be due to its phytotonic effect as reported by Jarande and Dethe [10] in cotton. Minimum suggested seed germination percentage in chickpea was 85 %. In the present investigation, seeds treated with imidacloprid (all three

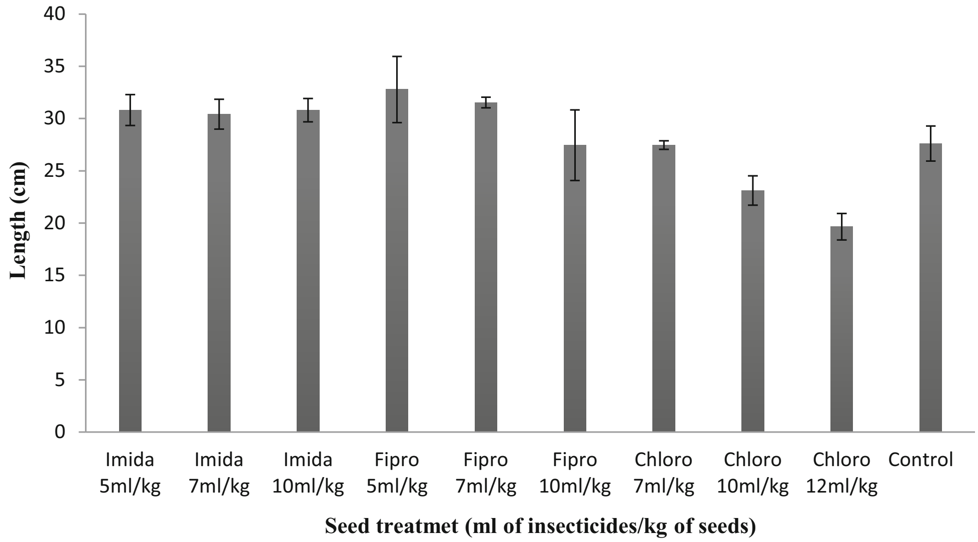


**Fig. 1** Effect of insecticidal seed treatment on chickpea seedling length

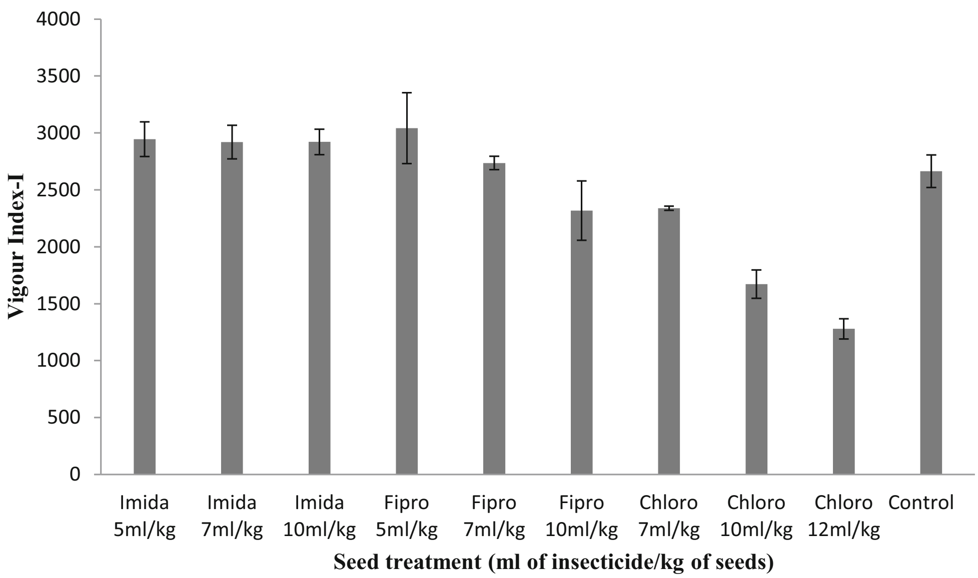


**Fig. 2** Effect of insecticidal seed treatment on vigour index-I in chickpea

test-doses 5, 7 and 10 ml/kg), fipronil (two doses 5 and 7 ml/kg) and chlorpyriphos (only one dose 6 ml/kg) showed the germination above 85 %. It is acceptable standard according to ISTA recommendation [7]. But chlorpyriphos treated seeds with two test-doses 10 and 12 ml/kg seed, the germination was 72.3 and 65 %, respectively. It was significantly lower than control that may be due to the toxic effect on some physiological and biochemical properties of seeds. All the three test doses of imidacloprid resulted in substantial increase in total length and vigour indices. Seed-treatment with chlorpyriphos (all three doses) resulted in substantial reduction in total length and vigour indices (Figs. 1, 2, Table 1). Vigour indices (both I and II) were observed to increase substantially in all test-doses of imidacloprid showing phytotonic effect on seedling growth, whereas, fipronil treatments (at two doses 5 and 7 ml/kg) showed increase in Vigour Index I only; however, all three doses resulted in reduced Vigour Index II.

### Rapid Tetrazolium Test

Untreated seeds (control) resulted in (1–2 %) dead seeds (Fig. 3). Chlorpyriphos at higher test-doses (i.e. 10, 12 ml/kg) resulted in higher dead seeds i.e. 16 and 19 %, respectively. Number of dead seeds also increased in high test-doses of fipronil but it was not as high as that of chlorpyriphos. Imidacloprid did not cause any harmful effect on viability of seeds as clear from the fact that dead seeds obtained were only 1–2 %. Overall, there was two-fold increase in dose, no phytotoxicity was obtained as there was negligible increase in dead seeds. The safety of imidacloprid is thus very much pronounced in the present investigation.

### Termiticidal Seed Treatments: Deleterious Impact

Rapid TZ test was done as per the guidelines (ISTA, 2007: International Seed Testing Rule, published by ISTA, Zurich, Switzerland) [7]. Deleterious impacts of chlorpyriphos seed treatment on the seeds was studied, in vitro, via light microscopy (LM). Seed tissue damage was evident from the death of radicles as evidenced by LM-study (Fig. 4). The present study suggests that much care must be taken while recommending chlorpyrophos as seed dressing.

### In vitro Compatibility Test for Insecticides and Biofungicide

In the in vitro compatibility test without seeds, observations were carried out on 24, 48, 72 and 96 h after incubation as growth of *T. harzianum* in control continues till 96 h. As Table 3 shows that the higher inhibition

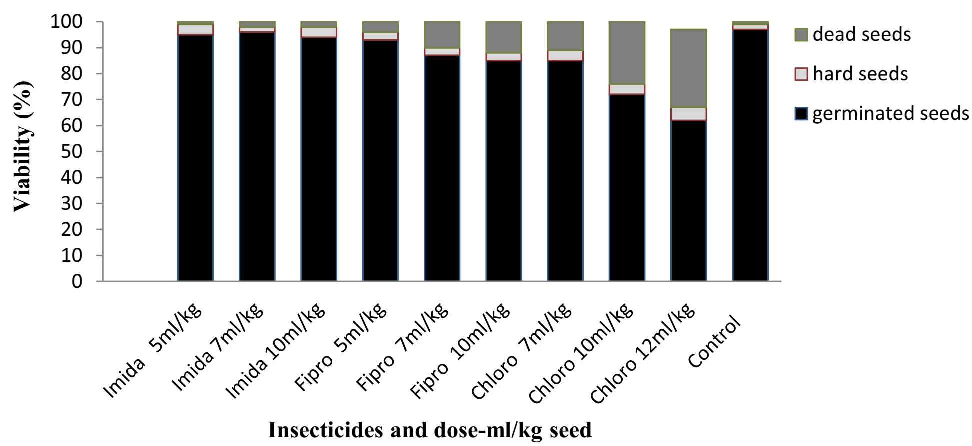


**Fig. 3** Effect of insecticidal treatment on chickpea seed viability

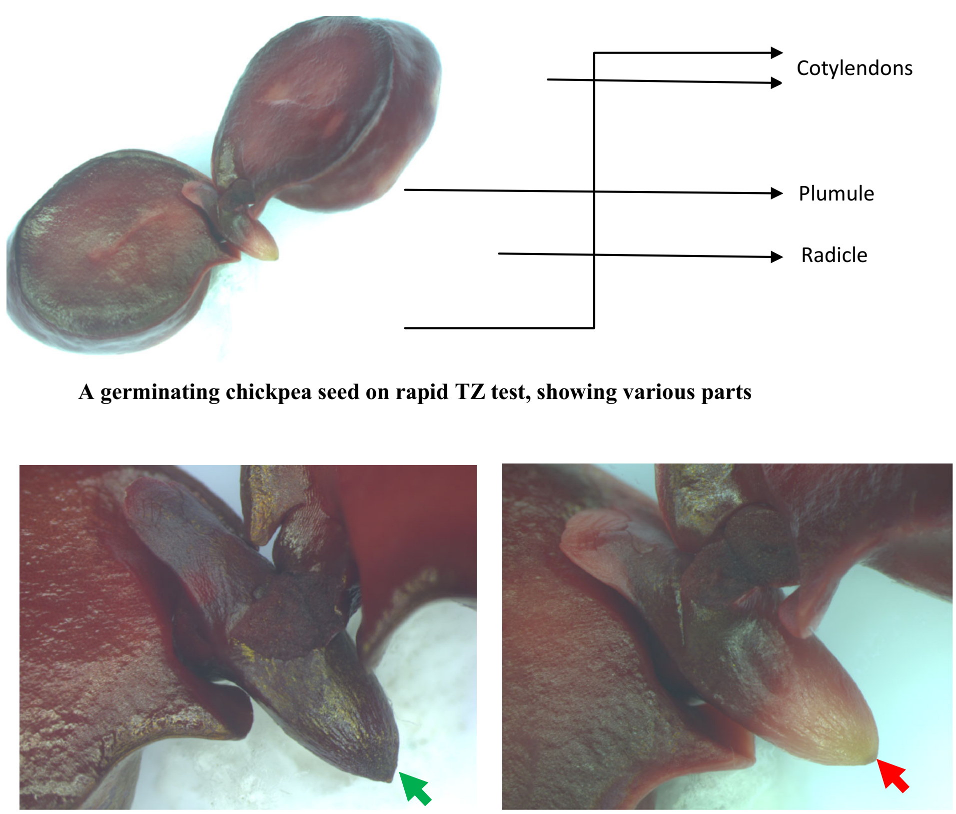


**Fig. 4** Live (*green arrow*) and dead tissues (*red arrow*) of radicle in seed-treatment (Light Microscopy study)

**Table 3** Effect of different termiticidal doses on *T. harzianum* on PDA medium 96 h after incubation

| Treatments | Treatment detail | Mean colony diameter (mm) | Inhibition (%) |
|---|---|---|---|
| $T_1$ | Control | 90.00[a] | 0 |
| $T_2$ | Imidachloprid (5 ml) | 88.66[ab] | 1.43 |
| $T_3$ | Imidachloprid (7 ml) | 87.33[b] | 2.96 |
| $T_4$ | Imidachloprid (10 ml) | 81.00[c] | 10.00 |
| $T_5$ | Fipronil (5 ml) | 80.66[c] | 10.37 |
| $T_6$ | Fipronil (7 ml) | 71.33[d] | 20.74 |
| $T_7$ | Fipronil (10 ml) | 61.66[e] | 31.48 |
| $T_8$ | Chlorpyriphos (7 ml) | 00.00[f] | 100 |
| $T_9$ | Chlorpyriphos (10 ml) | 00.00[f] | 100 |
| $T_{10}$ | Chlorpyriphos (12 ml) | 00.00[f] | 100 |

Figures having same letter as superscripts in a column indicate the values are not significantly different according the LSD at 0.05α

percentage of *T. harzianum* was recorded against all test-doses of chlorpyriphos as cent percent inhibition with 0 mm mean colony diameter (MCD). In case of imidachloprid, all test doses (5, 7 and 10 ml/kg seed) were found highly compatible with *T. harzianum* recording 1.43 % (88.66 mm MCD), 2.96 % (87.33 mm MCD) and 10 % (81 mm MCD) of growth inhibitions. All doses (5, 7 and 10 ml/kg seed) of fipronil were found moderately compatible with *T. harzianum* with 10.37 % (80.66 mm MCD), 20.74 % (71.33 mm MCD) and 31.48 % (61.66 mm MCD) (Table 3 and Fig. 5).

### Combinatorial Seed Treatments

Insecticides, biofertilizers (*Rhizobia*) and biofungicides (*Trichoderma*) are often required to be taken as seed treatments on field. In this combinatorial approach of compatibility assessment, effect of different combinations on the seedling growth and development was verified (Table 2). Germination of seeds in treatments which include imidacloprid $T_1$ (imidacloprid @ 5 ml/kg + *Mesorhizobium*) and $T_4$ (imidacloprid @ 5 ml/kg, *Mesorhizobium* and *Trichoderma*), recorded higher germination than that of control but it was statistically at par with control value. The treatments in which seeds were treated only with *Mesorhizobium* showed no compatibility problem. Treatments $T_2$ and $T_5$ (including fipronil) resulted in slightly decreased germination showing slight compatibility problem but it can be acceptable as germination is above ISTA specification (>85 %). Combinations which include chlorpyriphos resulted in decrease in germination percentage of seeds in addition to pale yellow colour of seedlings and reduced growth rate, which are statistically significant with control values and reveal major degree of incompatibility of chlorpyriphos with *Mesorhizobium* and *Trichoderma*.

In combinatorial seed treatments total length of seedling in control was 36.4 cm, but increased significantly in treatment $T_7$ (seed treated with only *Mesorhizobium*; 38.5 cm), $T_2$ (imidacloprid + *Mesorhizobium*) and $T_4$ (imidacloprid + *Mesorhizobium* + *Trichoderma*). In control, Vigour Index-I and II were 3510.31 and 70.95 respectively. Vigour indices (I and II) increased substantially in $T_1$ (3885.73 and 78.20), $T_4$ (3930.99 and 78.74) and $T_7$ (3680.55 and 76.16) indicating phytotonic effect of the treatments. In treatments $T_3$ and $T_6$ (including chlorpyriphos), drastic reduction in vigour indices (both I and II) were recorded. In all the above measured parameters, combination problem was the major factor in treatments which includes chlorpyriphos (Table 2).

### Root Nodulation Test: Pot Trial

In pulses, seed is often inoculated with adapted and efficient $N_2$-fixing strains of *Rhizobium* to ensure symbiotic efficiency for adequate nodulation and nitrogen fixation. Several workers reported that at recommended rate of application, these fungicides and insecticides have no suppressive effects on the growth parameters of legumes [11, 12]. On the other hand, fungicides and insecticides may adversely affect the legume—*Rhizobium* symbiosis, by inhibition of survival of the applied *Rhizobia* on seeds at the sowing time. This may lead to slow establishment of *Rhizobia* in the rhizosphere, which may reduce nodulation and $N_2$ fixation. Fox et al. [13] observed that pesticides chyrsin, methyl parathion, DDT, bisphenol A and pentachlorophenol reduced symbiotic efficiency of rhizobia in alfalfa.

Pot trial for root nodulation in chickpea showed increased root nodules in *Rhizobium* culture treated pulse seedlings (11 against 7.66 root nodules/seedling in control). Chlorpyriphos treatments (with *Rhizobium* and with *Rhizobium* + *Trichoderma*) exhibited detrimental effects on root nodules [$T_3 = 8.66$; $T_6 = 7.33$ as against $T_7$ (with

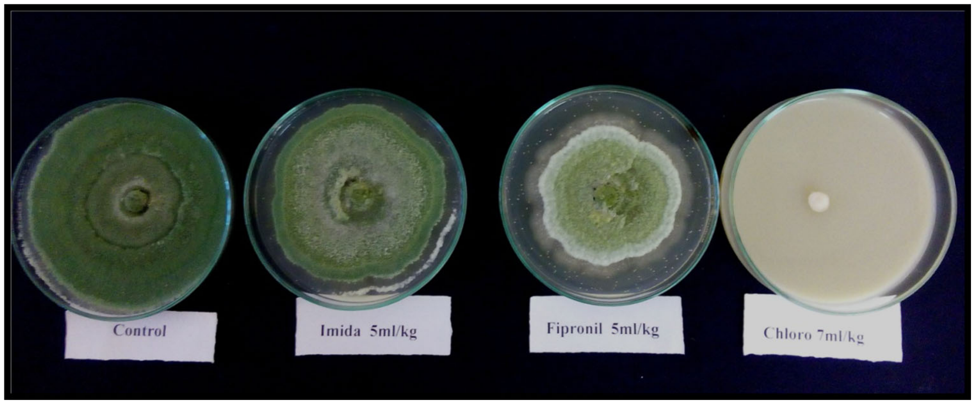


**Fig. 5** Biofungicide (*Trichoderma harzianum*) colony growth on PDA medium amended with insecticides after 96-h (Poison food technique)

biofertliser) = 11 root nodules/seedling]. Imidacloprid and fipronil both as termiticidal seed treatments (along with biofertilizer and biofungicide) exhibited better root nodulation (Fig. 6).

The reaction of seed treatment chemicals was reported in germination of various crops like groundnut [14], chickpea [15], green gram [16], safflower and sesame [17], and okra [18]. Suppression of germination and subsequent growth by the pesticidal treatments indicate that some of the biochemical processes are taking place during germination due to rapid imbibition rate, higher amount of seed leachate conductivity, higher seed respiration rate and higher intensity of dehydrogenase activity that reflect the vigour of seeds as affected by seed treatment which was experimentally described by Chaudhary et al. [19]. Chopra and Chandra [20] also reported almost similar findings for reduction in germination which was due to significant reduction in the formation of reducing sugars and free amino acids in mustard. Adverse effects of pesticides on seed germination were demonstrated by Scopes [21] and Gifford et al. [22]. Laboratory trials also indicated that chlorpyriphos (@ 9 ml/kg) significantly inhibited the wheat germination both for in vitro and pot culture [23]. The results of the present study revealed that seed-treatments with test-doses of fipronil @ 10 ml/kg and chlorpyriphos @ 12 ml/kg were found detrimental to the seedlings of the chickpea crop.

The present in vitro investigation revealed that higher dose of chlorpyriphos significantly affected the germination leading to drastic reduction in the germination as compared to control. As per the management package suggested by NCIPM, Govt. of India, 15–30 ml of chlorpyriphos (20EC)/kg of chickpea seed has been suggested for seed treatment [24], but the present experimental results do not corroborate with this suggestion. Chlorpyriphos is the common and most popular seed treatment practised widely in India unfortunately without any label claims. Chickpea and pigeon pea are predominantly grown on marginal or sub-marginal lands and by the resource poor farmers in India. As it is cheaper and easily available in common marketplace, it is quite popular, at the same time the toxic detrimental effect for this chemical has benn revealed. So field recommendations in developing countries must be given with appropriate precautions.

The compatibility and even an occasional synergism of *Rhizobium* + *Trichoderma* combination has been reported in chick pea [25]. The present study has included the insecticides in combination. Efficacy and compatibility of insecticides, fungicides and *Rhizobium* inoculant combination for seed treatment in chickpea was previously tested

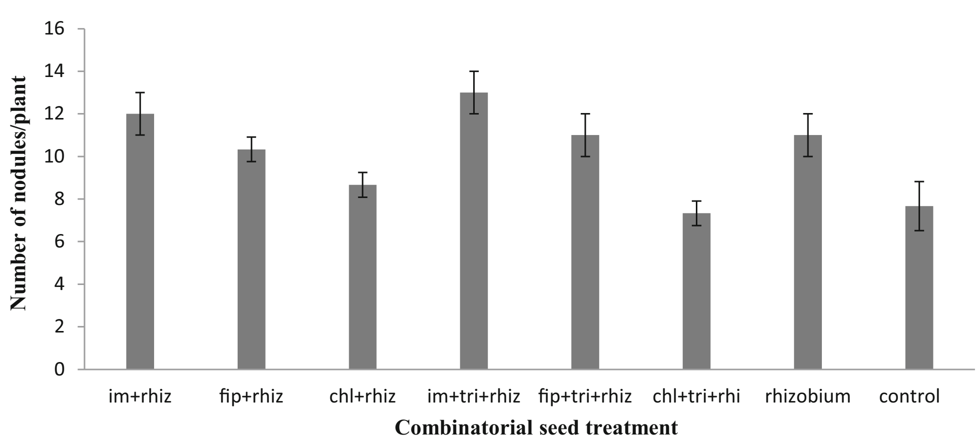


**Fig. 6** Effect of combinatorial seed treatments on root nodulation in chickpea

by Cheema et al. [26]. In their field experiments (2005–2008), endosulphan and chlorpyriphos were applied with recommended fungicide captan and rhizobium inoculant. Endosulfan @ 15 ml/kg and chlorpyriphos @ 10 ml/kg seeds had no adverse effect on germination, nodulation, yield-attributing characters and grain yield when applied alone or in combination with captan and *Rhizobium* inoculant, which indicated the compatibility of the tested insecticides. However, the present result is contrary to other results in which the authors have found that chlorpyriphos 20 EC @ 7 ml/kg causes detrimental effect on nodulation which resulted in reduced root nodulation.

Good Agricultural Practice (GAP) successfully reduces pesticide consumption without adversely affecting the agricultural productivity. This was facilitated by appropriate policies that discouraged pesticide use, and favoured IPM application. Total Seed Treatment Campaign of India is the noteworthy governmental policy in this aspect. However, it can be mentioned here that in real field situations the demands are different. Simply advocating insecticidal treatments to farmers may not cater to the real field demand. In conditions of multi-pests and diseases, combinatorial seed treatments may be required, for which the present investigation is a pioneer work for the farming community. An estimated loss of 5–15 % chickpea crop is reported alone by termites [24]. Insecticides included in the present recommendation are also termiticides, which can well be used against termites as seed treatment in addition to targeting sucking insect pests as well. Inclusion of the bio-fertilisers in combination schedule still makes the seed-treatment package, thus this instils realistic and relevant holistic approach in the context of Integrated Crop Management in chickpea.

## Conclusion

It may be inferred from the present study that the imidacloprid is having phytotonic effect on the growth of chickpea which might result into enhancement of all seedling parameters significantly higher than that of control. As far as compatibility of imidacloprid with biofungicide, *T. harzianum* and biofertilizer, *Mesorhizobium* is concerned, it reaches highest degree of compatibility. Higher doses of chlorpyriphos have phytotoxic effect on the growth of chickpea seedlings which results into reduction of all seedling parameters. Tetrazolium test inferred that higher doses of chlorpyriphos affected the growth of radicle, hence reduced the germination percentage by increasing the dead seeds in germination test. Successive increase in doses of imidacloprid along with biofungicide, *T. harzianum* and biofertilizer, *Mesorhizobium* results in maximum number of root nodules per plant. The present study indicates that imidacloprid can be effectively used as seed protectant along with biofertilizer *Mesorhizobium* spp. Fipronil at lower recommended dose can also be used along with both biofungicide and biofertilizer effectively. Chlorpyriphos, though cheapest, is not suitable either alone or in combination with biofungicide and biofertilizer as it hampers the growth of chickpea.

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### Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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