Indian Phytopath. 52 (2) : 174-176 (1999)

Integrated biological-chemical control of *Botrytis* gray mould of chickpea

M.P. HAWARE, P.K. MUKHERJEE[1], J.M. LENNE, S. JAYANTHI, H.S. TRIPATHI[2] and Y.P.S. RATHI[2]
International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324

Key words : *Botrytis cinerea, Trichoderma viride,* biocontrol, integrated control, chickpea, vinclozolin

Botrytis gray mould (BGM), caused by *Botrytis cinerea* Pers. ex. Fr., is an important disease of chickpea (*Cicer arietinum* L.) in several parts of India, Nepal, Bangladesh and Pakistan causing 70-100% yield losses under favourable conditions (5,7). It has also been reported from Myanmar, Argentina, Australia, Canada and Chile. In the absence of resistant chickpea genotypes, growers rely on fungicides like vinclozolin and carbendazim, but fungicides alone are ineffective if climatic conditions favour infection (7,8). Development of resistance in *B. cinerea* to these fungicides is very common and widespread, thereby reducing their effective period of use (4,13).

Control of *B. cinerea* using the antagonistic fungi *Trichoderma* spp. has shown great promise including commercial successes in several greenhouse crops and orchards (1,2,6,12). We have recently reported biocontrol potential of *T. viride* isolate T-15 (isolated by us from chickpea rhizosphere) on *B. cinerea* in chickpea under controlled environmental conditions (10,11). However, to our knowledge, there is no report on biological control of this pathogen on a field crop under the field conditions, except a recent report published by us (9). Integration of a lower dose of fungicide with biocontrol has been suggested by various workers in order to enhance the performance of the biocontrol system as well as to reduce the probability of development of fungicide resistance in this pathogen (3,14). In our earlier efforts, we obtained some vinclozolin-resistant strains of *T. viride* which could be successfully integrated with the fungicide in growth room studies (11). In the present paper, successful integrated biological-chemical control of BGM of chickpea under field conditions using a vinclozolin-resistant strain of *T. viride* has been reported.

T. viride strain T-15.4, resistant to vinclozolin, was obtained by selection of the wild type isolate T-15 on 500 mg l^{-1} of vinclozolin (Ronilan® 50 WP, BASF, Ludhingshafen) as described previously (11). The fungus was cultured and maintained on potato dextrose agar at 25±1°C. For field application, the fungus was multiplied on potato dextrose broth pH 6 (50 ml medium in 250 ml Erlenmeyer flasks) for 7 days under 12 h light cycle without shaking. The culture was harvested on muslin cloth and blended in sterile distilled water. The spore concentration was adjusted to 10^8 ml^{-1} (mycelial fragments were also present).

Field experiments were conducted at the International BGM Screening Nursery located at the Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar (29°N,79°30'E) during 1994-95 and 1995-96 post-rainy seasons. One plot of 3 m × 1.8 m constituted one experimental unit. Plot to plot distance was 1.5 m and row to row 45 cm. Seed rate was 70 kg ha^{-1}. Chickpea cultivar H-208 which is highly susceptible to BGM was used as the test variety. The design of experiment was randomised block design with 4 replicates. In both 1994-95 and 1995-96 field experiments, treatments consisted of control (no spray), three sprays of *T. viride* at 20 days intervals, three sprays of Ronilan® (0.2%) at 20 days intervals (recommended dose), and a treatment consisting of a tank mix of *T. viride* (5×10^7 spores ml^{-1}) and Ronilan® (0.1%), followed by *T. viride* alone, followed by *T. viride*-Ronilan® (0.1%) mix at 20 days intervals. Carboxymethyl cellulose (0.5%) was used with *T. viride* spore suspension as an inoculum carrier (14). First spray was applied at the first sight of the symptom (natural incidence of the disease, no spray of the pathogen applied). Spraying was done until run-off using a conventional hand compression sprayer. The sprayer was washed thoroughly between two treatments to avoid cross-contamination. Disease severity was scored before harvesting on a 1-9 point rating scale where 1=

[1]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085; [2]G.B. Pant University of Agriculture and Technology, Pantnagar, Uttar Pradesh 263 145.

no infection and 9= plants killed. Data on grain yield was recorded after harvesting.

In 1994-95 field experiment, three applications of *T. viride* (5×10^7 spores ml^{-1}) reduced the disease severity by 20.7% and increased the yield by 33% (Table 1). Ronilan® (0.2%, sprayed thrice) was as effective as the integrated treatment with reduced dose of the fungicide: both the treatments reduced the disease severity by 31% and increased the yield by 42 - 50%. Similar trend was observed in 1995-96 crop season also. Three applications of *T. viride* reduced the disease and increased the yield by 28.6 and 37.5% respectively (Table 1). Integration with a reduced application of Ronilan® (0.1%, twice) improved the performance of *T. viride* by reducing the disease severity and increasing the yield by 45.7 and 75%, respectively, which was statistically same as observed with three applications of (0.2%) Ronilan® alone.

Table 1. Integrated control of Botrytis gray mold of chickpea under field conditions during 1994-95 and 1995-96 crop seasons

Treatments	1994-95 Disease rating*	1994-95 Yield (t/ha)	1995-96 Disease rating	1995-96 Yield (t/ha)
Control	5.8[a]	1.2[a]	7.0[a]	0.8[a]
TV→TV→TV[X]	4.6[b]	1.6[b]	5.0[b]	1.1[b]
RN→RN→RN[Y]	4.0[c]	1.8[c]	3.3[c]	1.5[c]
$RN_{1/2}$+TV→TV→ $RN_{1/2}$+TV[Z]	4.0[c]	1.7[bc]	3.8[c]	1.4[c]

*Disease was scored on a 1-9 point scale where 1 = no symptom and 9 = plants killed; Data are the mean of 4 replicates; Data in the same column followed by the same letters are not significantly ($P\leq0.05$) according to Duncan's multiple range text.

[X]Three applications of *Trichoderma viride* (5×10^7 spores ml^{-1}) at 20 day intervals.

[Y]Three applications of Ronialn® (0.2%) at 20 day intervals.

[Z]One application of "*T. viride* + Ronilan® (0.1%)", followed by one application of *T. viride*, then one application of "*T. viride* + Ronilan® (0.1%)", at 20 day intervals.

B. cinerea has been difficult to manage mainly due to the absence of resistance in the chickpea germplasm, and due to the rapid development of resistance in the pathogen to the recommended fungicides. The present experiments were designed to assess the performance of a vinclozolin-resistant strain of *T. viride* applied alone or combined with lower dose of vinclozolin in suppressing BGM of chickpea under field conditions using conventional spraying practices. The results indicate that in combination with *T. viride*, the requirement of fungicide could be reduced to one third (0.1 % twice, compared to 0.2% thrice) of the recommended dose. The results are significant not only because it exhibits the potential of a biocontrol system of a foliar disease in a tropical country under natural conditions of disease development, but also because this is the first successful attempt to control *B. cinerea* in a field crop where the microclimate is entirely different from greenhouse and horticultural crops where all previous successes in using *Trichoderma* spp. have been reported (2,6,12).

The treatment schedule vinclozolin + *T. viride* followed by *T. viride* alone, and then vinclozolin + *T. viride* was followed in this experiment with the objective of reducing the chance of development of fungicide resistance in the pathogen. This could be possible in two ways: by reducing the fungicide requirement to one third, thus reducing the selection pressure, and by combining and rotating with a non-selective biofungicide (*T. viride*) with several modes of action against plant pathogenic fungi.

REFERENCES

1. **Elad, Y.** (1994). *Crop Prot.* **13** : 35-38.
2. **Elad, Y., Malathrakis, N.E. and Dik, A.J.** (1996). *Crop Prot.* **15**: 229-240.
3. **Elad, Y., Zimand, G., Zaqs, Y., Znriel, S. and Chet, I.** (1993). *Plant Pathol.* **42**: 324-332.
4. **Faretra F. and Pollastro S.** (1993). *Plant Pathol.* **42**: 48-57.
5. **Grewal, J.S. and Laha, S.K.** (1983). *Indian Phytopath.* **36** : 516-520.
6. **Harman, G.E., Latorre, B., Agoson, E., San Martin, R. Riegel, D.G., Nielson, P.A., Tronsmo, A. and Pearson, R.C.** (1996). *Biol. Cont.* **7**: 269-266.
7. **Haware, M.P. and McDonald, D.** (1992). In: *Botrytis Gray Mold of Chickpea : Summary Proceedings of the BARI/ICRISAT Working Group Meeting,* 4-8 March 1991, Joydebpur, Bangladesh (Eds., Haware, M.P., Faris, D.G. and Gowda, C.L.L.). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, pp 3-6.
8. **Haware, M.P. and McDonald, D.** (1993). In: *Botrytis Gray Mold of Chickpea: Recent Advances in Research, Summary Proceedings of the Second Working Group Meeting,* 14-17 March 1993, Rampur, Nepal (Eds., Haware, M.P., Gowda, C.L.L. and McDonald, D.). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, pp. 3-6.

9. **Haware, M.P., Lenne, J.M. and Mukherjee, P.K.** (1996). *Proceedings: XI International Botrytis Symposium,* 23-27 June 1996, Wageningen, p.98 (Abstract).

10. **Mukherjee, P.K. and Haware, M.P.** (1993). *Intl. Chickpea Newsl.* **28**: 14-15.

11. **Mukherjee, P.K., Haware, M.P. and Jayanthi, S.** (1995). *Indian Phytopath.* **48**: 141-149.

12. **O'Neill, T.M., Elad, Y., Shtienberg, D. and Cohen, A.** (1996). *Biocont. Sci. Tech.* **6** : 139-146.

13. **Pommer, E.H. and Lorenz, G.** (1982). *Crop Prot.* **1** : 221-230.

14. **Tronsmo, A.** (1991). *Biol. Cont.* **1** : 59-62.

Received for publication August 24, 1998