IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Screening Of Fungal Isolates From Rice Weeds For Phytotoxicity And Mycoherbicidal PotentialIn Chhattisgarh, India

Tarendra Kumar Sahu 1, Dr. Sushma Dubey1*

Department of Biotechnology Kalinga University Naya Raipur Chhattisgarh,Pin 492101 India

ABSTRACT

Weeds such as *Echinochloa crus-galli*, *Cyperusrotundus*, *Cynodondactylon*, and *Leptochloachinensis* are among the most problematic species in rice cultivation, leading to significant yield losses and increasing reliance on chemical herbicides. The objective of this study was to identify indigenous fungal pathogens with strong mycoherbicidal potential for eco-friendly weed management in rice ecosystems.A comprehensive field survey was conducted in four major rice-growing districts of Chhattisgarh—Raipur, Dhamtari, Gariyaband, and Balod—to collect diseased weed tissues and rhizospheric soils. Forty fungal isolates were recovered, purified, and identified based on morphological characteristics. Pathogenicity tests were carried out under controlled conditions on healthy seedlings of the target weeds using spore suspensions (1×10^6 spores/mL). Disease severity was assessed through visible symptoms such as necrosis, chlorosis, wilting, and blight and the most virulent strains were selected based on a standard rating scale.Among the tested isolates, *Fusariumoxysporum* (FSRW#04) demonstrated the highest pathogenicity, causing severe symptoms in all four weed species while showing negligible phytotoxic effects on rice seedlings. The findings suggested that *F. oxysporum* is a promising candidate for development as a biological weed control agent. Further studies on formulation and field validation are recommended to assess its scalability and environmental safety.

Keywords: *Fusariumoxysporum*, Mycoherbicide, Rice weed control, *Echinochloa crus-galli*, *Cyperusrotundus*, Fungal screening, Biological control.

Introduction: Weeds pose a significant challenge in rice cultivation by competing for essential resources such as water, nutrients, and sunlight, leading to substantial yield losses (Ghosh & Das, 2017). Chemical herbicides have long been the primary solution for weed management; however, their overuse has resulted in several agronomic and ecological concerns, including the development of herbicide-resistant

weed species, soil and water contamination, and adverse effects on non-target flora and fauna (Prasad &Yadav, 2018). Furthermore, the effectiveness of synthetic herbicides has been declining in certain cases, and their non-biodegradable nature adds long-term toxicity to the agroecosystem. Previous studies on weed control have largely focused on synthetic chemical approaches, with limited exploration into biocontrol-based weed suppression strategies under diverse field conditions.

As a sustainable alternative, biological control using mycoherbicides—fungal-based bioherbicides—has gained increasing attention. Mycoherbicides are derived from naturally occurring fungal pathogens that infect specific weeds, thereby reducing their competitiveness without harming crops or beneficial organisms (Hoagland, 2001; Varejão et al., 2013). These fungi release phytotoxic secondary metabolites that interfere with physiological processes like nutrient transport and cell integrity, causing wilting, necrosis, and plant death. Among various fungi, genera such as *Fusarium*, *Alternaria*, and *Colletotrichum* have shown potential for mycoherbicidal development. However, many of these have not been fully tested against a wide spectrum of dominant weeds in tropical rice-growing regions.

The present study addresses this gap by conducting a primary screening of fungal pathogens isolated from naturally infected weed species in major rice-growing districts of Chhattisgarh. Forty isolates were evaluated for their pathogenicity against four major weeds—*Echinochloa crus-galli*, *Cyperusrotundus*, *Cynodondactylon*, and *Leptochloachinensis*. Among them, *Fusariumoxysporum* emerged as the most virulent and host-specific isolate, demonstrating strong phytotoxicity while being non-damaging to rice seedlings. This study aims to lay the groundwork for developing *F. oxysporum* as a potential mycoherbicide, contributing to integrated weed management strategies for more sustainable rice cultivation (Boyetchko et al., 2002; Pandey et al., 2018).

MATERIAL AND METHODS

1. Field Survey and Sample Collection

A field survey was conducted in four major rice-growing districts of Chhattisgarh—Raipur, Dhamtari, Gariyaband, and Balod—selected due to their high weed pressure and favorable agro-ecological conditions. Weeds exhibiting visible disease symptoms such as leaf spots, sheath rot, blight, and wilting were collected from naturally infested sites. The dominant target weed species included *Echinochloa crus-galli*, *Cynodondactylon*, *Cyperusrotundus*, and *Leptochloachinensis*. Symptomatic plant parts (leaves, stems, and roots) were excised and placed in sterile polythene bags, then transported to the laboratory under aseptic conditions for pathogen isolation (Barnett & Hunter, 1998).

2. Isolation of Fungal Pathogens

2.1 From Plant Tissues

Diseased plant tissues were surface sterilized with either 0.1% mercuric chloride or 70% ethanol for 30–60 seconds, followed by triple rinsing in sterile distilled water (Agrios, 2005). Small segments (approximately 0.5–1.0 cm^2) were excised from the lesion margins and aseptically placed on Potato

Dextrose Agar (PDA) plates. Plates were incubated at 25–28°C for 5–7 days. Distinct fungal colonies were sub-cultured to obtain axenic (pure) cultures for further analysis (Waller et al., 2008).

2.2 From Rhizospheric Soil

Soil samples were collected from the rhizosphere of the infected weeds using two methods:

- **Serial Dilution Method**: 10 g of soil was suspended in 90 mL sterile distilled water and serially diluted up to 10^{-6}. Aliquots of 1 mL were plated on PDA supplemented with streptomycin (to suppress bacterial growth).
- **Soil Plate Method**: 0.5–1.0 g of rhizospheric soil was evenly sprinkled over the surface of PDA plates.

Plates were incubated at 25–28°C for 5–7 days, and emerging fungal colonies were isolated and purified (Nene &Thapliyal, 2000; Aneja, 2003).

3. Identification of Pathogens

Fungal isolates were identified based on their macroscopic (colony texture, pigmentation) and microscopic (spore morphology, septation, and sporulation) characteristics. Identification was aided by standard mycological keys, monographs, and published manuals (Ellis, 1971).

4. Pathogenicity Testing and Virulence Assay

The virulence of each isolate was evaluated on healthy weed seedlings (3–4 leaf stage) grown under controlled environmental conditions. Spore suspensions (1×10^6 spores/mL) were prepared in sterile distilled water. Inoculation was performed by foliar spray, stem injection, or root-dip methods depending on the weed species. The inoculated plants were placed in growth chambers at 25 ± 2°C with 80–90% relative humidity and observed for disease symptoms over 7–14 days. Symptoms were scored using a standardized disease severity scale. Koch's postulates were fulfilled by re-isolating the pathogen from newly infected tissues and comparing morphological features with the original isolates (Agrios, 2005).

5. Selection of Virulent Strains

Forty fungal isolates were tested to ensure a broad representation of pathogen diversity from different ecological niches and weed hosts. This number was chosen to balance the need for diverse screening with the feasibility of conducting controlled bioassays. Isolates that caused severe disease symptoms—such as necrosis, wilting, or blight—were classified as highly virulent. Among them, *Fusarium oxysporum* consistently exhibited the highest phytotoxicity against all four major weed species and was selected for further bioherbicidal evaluation (Boyetchko, 2000; Hoagland, 2001)

RESULTS

Forty fungal isolates were successfully recovered from infected tissues and rhizospheric soils of predominant rice weed species across the surveyed regions of Chhattisgarh. These isolates were identified based on morphological characteristics and pathogenic symptoms associated with each fungus. The pathogens were isolated from various plant parts exhibiting typical disease symptoms such as blast, sheath blight, root rot, seed mold, leaf blight, and anthracnose, as well as from rhizospheric soils.

In table 1 among the isolates, Magnaporthe oryzae (FSRW#01), Rhizoctonia solani (FSRW#02), and Sarocladium oryzae (FSRW#03) were prominent foliar and sheath pathogens, while Fusarium spp.

(FSRW#04 to FSRW#07) were frequently associated with root, stem, and seed infections. A diverse range of seed-borne fungi including *Aspergillus*, *Penicillium*, and *Alternaria* species was also recorded, many of which are known mycotoxin producers(Pitt & Hocking, 2009. Notably, Trichoderma spp. (FSRW#20–21) were recovered from the rhizosphere and are recognized for their biocontrol potential (Harman et al., 2004).

The presence of both pathogenic and saprophytic fungi in different ecological niches highlights the complexity of the mycoflora associated with rice weed plants. These findings provide a foundation for further pathogenicity testing and selection of virulent strains for potential use in mycoherbicide development(Charudattan, 2001).

Table 1. Fungal isolates recovered from infected rice weed plant parts and rhizospheric soil

S. No.	Fungal Name	Isolate No.	Source / Symptoms
1	*Magnaporthe oryzae*	FSRW#01	Blast (leaf, neck, node)
2	*Rhizoctonia solani*	FSRW#02	Sheath blight
3	*Sarocladium oryzae*	FSRW#03	Sheath rot
4	*Fusarium oxysporum*	FSRW#04	Root rot, wilting
5	*Fusarium solani*	FSRW#05	Stem rot, root infection
6	*Fusarium moniliforme*	FSRW#06	Seed rot, mycotoxins
7	*Fusarium verticillioides*	FSRW#07	Root colonizer, seed infection
8	*Helminthosporium oryzae*	FSRW#08	Brown spot
9	*Drechslera oryzae*	FSRW#09	Leaf & sheath spot
10	*Curvularia lunata*	FSRW#10	Leaf blight
11	*Curvularia pallescens*	FSRW#11	Leaf and stem lesions
12	*Alternaria alternate*	FSRW#12	Leaf spot, seed infection
13	*Alternaria padwickii*	FSRW#13	Grain discoloration
14	*Aspergillus flavus*	FSRW#14	Seed mold, aflatoxins
15	*Aspergillus niger*	FSRW#15	Seed rot, storage fungi
16	*Aspergillus terreus*	FSRW#16	Rhizosphere colonizer
17	*Aspergillus fumigates*	FSRW#17	Seed colonization
18	*Penicillium citrinum*	FSRW#18	Seed mold, mycotoxin producer
19	*Penicillium funiculosum*	FSRW#19	Rhizosphere fungi
20	*Trichoderma harzianum*	FSRW#20	Biocontrol agent (soil)
21	*Trichoderma viride*	FSRW#21	Biocontrol agent (antagonist)
22	*Sclerotium rolfsii*	FSRW#22	Collar rot
23	*Sclerotinia sclerotiorum*	FSRW#23	White mold (under wet conditions)
24	*Cladosporium herbarum*	FSRW#24	Leaf colonizer, secondary infection
25	*Cladosporium cladosporioides*	FSRW#25	Leaf surface fungi

26	*Phoma sorghina*	FSRW#26	Seed rot
27	*Phoma herbarum*	FSRW#27	Stem blight
28	*Colletotrichum gloeosporioides*	FSRW#28	Anthracnose-like symptoms
29	*Colletotrichum graminicola*	FSRW#29	Petiole/stem infection
30	*Cercospora oryzae*	FSRW#30	Leaf blight
31	*Cercospora* sp.	FSRW#31	Leaf lesion
32	*Myrothecium verrucaria*	FSRW#32	Leaf necrosis
33	*Macrophomina phaseolina*	FSRW#33	Root and stem rot
34	*Pythium ultimum*	FSRW#34	Damping off
35	*Glomerella graminicola*	FSRW#35	Seed rot
36	*Mucor hiemalis*	FSRW#36	Leaf, seed mold
37	*Nigrospora oryzae*	FSRW#37	Leaf tip dieback
38	*Bipolaris oryzae*	FSRW#38	Brown spot
39	*Epicoccum nigrum*	FSRW#39	Rhizosphere fungi
40	*Humicola grisea*	FSRW#40	Soil-inhabiting decomposer

Table 2 presents the phytotoxic effects of Mycelial-Free Fungal Extracts (MFFE) derived from 40 fungal isolates against four dominant rice weeds—*Cyperus rotundus* (CR), *Echinochloa crus-galli* (EC), *Cynodon dactylon* (CD), and *Leptochloa chinensis* (LC)—using Whole Plant Bioassay (WPB), Shoot Cut Bioassay (SCB), and Petiole Plant Bioassay (PPB). The table reports percent phytotoxic damage (e.g., chlorosis, necrosis, desiccation, wilting) observed on each weed species post-treatment. Isolates such as *Fusarium oxysporum* (FSRW#04), *Drechslera oryzae* (FSRW#09), *Myrothecium verrucaria* (FSRW#32), *Bipolaris oryzae* (FSRW#38), and *Colletotrichum graminicola* (FSRW#29) recorded consistently high damage percentages across all weed species, earning a rating of 5, and are considered strong candidates for bioherbicidal applications. In contrast, isolates like *Trichoderma harzianum* (FSRW#20), *T. viride* (FSRW#21), and *Humicola grisea* (FSRW#40) showed minimal phytotoxicity, suggesting their primary role may be in biocontrol or decomposition rather than weed suppression. This rating system (1–5) helps categorize the potential of each fungal strain based on overall average damage across tested weeds.

Table 3 illustrates the dose-dependent phytotoxic effects of Mycelial-Free Fungal Extracts (MFFE) from eight fungal isolates tested against four major rice weeds: *Cyperus rotundus* (CR), *Echinochloa crus-galli* (EC), *Cynodon dactylon* (CD), and *Leptochloa chinensis* (LC). Each fungal extract was applied at four concentrations (25%, 50%, 75%, and 100%), along with a control (0%) to assess phytotoxicity, quantified as percent damage. Among the tested isolates, **Fusarium oxysporum (FSRW#04)** showed the **highest phytotoxic activity**, reaching up to **91–94% damage** at 100% concentration. Similarly, *Myrothecium verrucaria* (FSRW#32, #33) and *Bipolaris oryzae* (FSRW#38) also demonstrated strong weed suppression effects, particularly at higher concentrations. In contrast, *Colletotrichum graminicola*

(FSRW#29) and *Helminthosporium oryzae* (FSRW#08) exhibited moderate, yet consistent, phytotoxicity across all tested weed species. All fungi showed negligible effects in the control group, validating the specific impact of their bioactive metabolites.

Table 2. Phytotoxicity of Mycelial Free Fungal Extracts (MFFE) of Fungal Strains on Major Rice Weeds

S.N.	Fungal Name	Isolate No.	WPB/SCB/PPB Phytotoxic damage(%)				
			CR	EC	CD	LC	Overall Rating
1	*Magnaporthe oryzae*	FSRW#01	55	46	59	62	4
2	*Rhizoctonia solani*	FSRW#02	58	62	75	63	4
3	*Sarocladium oryzae*	FSRW#03	44	48	57	65	3
4	*Fusarium oxysporum*	FSRW#04	65	69	77	79	5
5	*Fusarium solani*	FSRW#05	49	52	63	59	3
6	*Fusarium moniliforme*	FSRW#06	40	45	58	58	3
7	*Fusarium verticillioides*	FSRW#07	42	48	60	66	3
8	*Helminthosporium oryzae*	FSRW#08	64	65	70	69	5
9	*Drechslera oryzae*	FSRW#09	65	68	78	73	5
10	*Curvularia lunata*	FSRW#10	58	60	72	75	4
11	*Curvularia pallescens*	FSRW#11	54	57	70	64	4
12	*Alternaria alternata*	FSRW#12	50	55	65	68	3
13	*Alternaria padwickii*	FSRW#13	48	52	60	69	3
14	*Aspergillus flavus*	FSRW#14	36	38	50	59	2
15	*Aspergillus niger*	FSRW#15	40	42	55	84	3

16	*Aspergillus terreus*	FSRW#16	35	38	48	44	2
17	*Aspergillus fumigates*	FSRW#17	38	42	54	58	2
18	*Penicillium citrinum*	FSRW#18	33	37	45	43	2
19	*Penicillium funiculosum*	FSRW#19	30	35	42	80	2
20	*Trichoderma harzianum*	FSRW#20	20	22	28	67	1
21	*Trichoderma viride*	FSRW#21	22	25	30	77	1
22	*Sclerotium rolfsii*	FSRW#22	55	60	68	45	4
23	*Sclerotinia sclerotiorum*	FSRW#23	59	63	75	28	4
24	*Cladosporium herbarum*	FSRW#24	48	50	60	30	3
25	*Cladosporium cladosporioides*	FSRW#25	45	48	58	68	3
26	*Phoma sorghina*	FSRW#26	43	47	56	75	3
27	*Phoma herbarum*	FSRW#27	46	50	60	60	3
28	*Colletotrichum gloeosporioides*	FSRW#28	60	65	78	58	5
29	*Colletotrichum graminicola*	FSRW#29	62	68	80	56	5
30	*Cercospora oryzae*	FSRW#30	58	61	70	60	4
31	*Cercospora sp.*	FSRW#31	56	60	67	78	4
32	*Myrothecium verrucaria*	FSRW#32	65	69	82	80	5
33	*Macrophomina phaseolina*	FSRW#33	64	67	78	70	5
34	*Pythium ultimum*	FSRW#34	51	55	66	67	4

35	*Glomerella graminicola*	FSRW#35	55	56	60	63	4
36	*Mucor hiemalis*	FSRW#36	30	35	42	78	2
37	*Nigrospora oryzae*	FSRW#37	40	43	55	66	3
38	*Bipolaris oryzae*	FSRW#38	67	70	84	28	5
39	*Epicoccum nigrum*	FSRW#39	34	36	45	30	2
40	*Humicola grisea*	FSRW#40	25	30	38	68	1

Note: CR – *Cyperus rotundus*, EC – *Echinochloa crus-galli*, CD – *Cynodon dactylon*, LC– *Leptochloa chinensis*,

Overall Rating is based on a 1–5 scale: 5 = Highly Phytotoxic, 1 = Weakly Phytotoxic.

Statistical validation (e.g., ANOVA, LSD) pending inclusion.

Table 3. Dose-Dependent Phytotoxicity of Top-Rated Fungal MFFE on Rice Weeds

S. No.	Culture No.	Fungus Name	MFFE con.	Phytotoxic Damage (%)			
				CR	**EC**	**CD**	**LC**
1.	FSRW#04	*Fusarium oxysporum*	Control	0	0	0	0
			25%	58	64	65	68
			50%	69	74	70	75
			75%	80	85	84	85
			100%	91	90	94	90
2.	FSRW#08	*Helminthosporium oryzae*	Control	0	0	0	0
			25%	35	36	45	44
			50%	42	46	55	54
			75%	62	55	65	60
			100%	68	61	67	60
3.	FSRW#09	*Drechslera oryzae*	Control	0	0	0	0
			25%	51	42	39	40

			50%	55	50	47	48
			75%	60	50	55	57
			100%	65	55	67	65
4.	FSRW#28	*Colletotrichum gloeosporioides*	Control	0	0	0	0
			25%	54	42	56	45
			50%	60	55	64	52
			75%	67	62	70	60
			100%	75	76	74	66
5.	FSRW#29	*Colletotrichum graminicola*	Control	0	0	0	0
			25%	35	32	29	28
			50%	50	45	40	47
			75%	57	55	51	57
			100%	65	61	68	64
6.	FSRW#32	*Myrothecium verrucaria*	Control	0	0	0	0
			25%	32	36	30	41
			50%	52	45	45	55
			75%	65	62	60	69
			100%	75	71	76	70
7.	FSRW#33	*Myrothecium verrucaria*	Control	0	0	0	0
			25%	40	43	31	36
			50%	52	51	50	49
			75%	65	63	56	60
			100%	85	59	72	75
8.	FSRW#38	*Bipolaris oryzae*	Control	0	0	0	0

			25%	43	41	47	43
			50%	59	55	53	55
			75%	67	60	62	67
			100%	75	68	73	72

Note: Values represent percent phytotoxic damage at each MFFE concentration. Each treatment should be replicated thrice, and statistical significance (e.g., $LSD_{5}\%$, SE±) should be computed for each parameter to confirm variability.

DISCUSSION

The screening of 40 fungal isolates revealed that *Fusarium oxysporum* (FSRW#04) was the most effective in suppressing major rice weed species, causing up to 94% phytotoxic damage at higher concentrations. Its strong bioherbicidal potential can be attributed to the production of phytotoxic metabolites like fusaric acid and its ability to colonize vascular tissues, disrupting water and nutrient flow. These findings are in line with earlier studies highlighting *F. oxysporum* as a potent biological control agent due to its broad-spectrum pathogenicity and systemic infection mechanisms.

Other fungi such as *Myrothecium verrucaria*, *Bipolaris oryzae*, and *Colletotrichum graminicola* also showed moderate to high phytotoxic effects, indicating their potential for further evaluation. In contrast, isolates like *Trichoderma harzianum* exhibited minimal toxicity, supporting their role more in biocontrol than in direct weed suppression. Overall, *F. oxysporum* demonstrates significant promise as a mycoherbicide for sustainable weed management in rice ecosystems, though field validation and formulation development are necessary for practical application.

CONCLUSION

The study demonstrated that Mycelial-Free Fungal Extracts (MFFEs) of *Fusarium oxysporum* exhibited strong, dose-dependent phytotoxic effects against dominant rice weeds such as *Cyperus rotundus*, *Echinochloa crus-galli*, *Cynodon dactylon*, and *Leptochloa chinensis*. These findings suggest that *F. oxysporum* holds significant potential as a bioherbicidal agent for sustainable and eco-friendly weed management in rice cultivation systems.

However, the current research was limited to laboratory-based assays, and further investigations are necessary to validate the efficacy under field conditions. Future studies should focus on large-scale field trials, formulation development, mode-of-action studies, and safety assessments on non-target organisms and rice crops to ensure practical application and environmental safety of *F. oxysporum* as a mycoherbicide.

ACKNOWLEDGEMENT

The Authors acknowledge their sincere thanks towards the Supervisor, Dr. Sushma Dubey, HOD, Dept. of Biotechnology, Kalinga University, Raipur for providing all the Laboratory Research facilities along with the technical support.

REFERENCES

- Agrios, G. N. (2005). *Plant Pathology* (5th ed.). Elsevier Academic Press.
- Aneja, K. R. (2003). *Experiments in Microbiology, Plant Pathology and Biotechnology*. New Age International.
- Barnett, H. L., & Hunter, B. B. (1998). *Illustrated Genera of Imperfect Fungi* (4th ed.). APS Press.
- Boyetchko, S. M., Rosskopf, E. N., & Caesar, A. J. (2002). Microbial weed control: research needs and recent progress. *Weed Research*, 42(1), 71–80.
- Charudattan, R. (2001). Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. *BioControl*, 46(2), 229–260.
- Dayan, F. E., Duke, S. O., &Romagni, J. G. (2000). Biological activity of allelochemicals. *Weed Science*, 48(6), 720–729.
- Ellis, M. B. (1971). *DematiaceousHyphomycetes*. Commonwealth Mycological Institute, Kew.
- Ghosh, S., & Das, S. (2017). Mycoherbicides: a new weapon for weed management. *International Journal of Agriculture Sciences*, 9(3), 3761–3766.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., &Lorito, M. (2004). Trichoderma species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2(1), 43–56.
- Hoagland, R. E. (2001). Mycoherbicides and natural phytotoxins as herbicides. In *Biologically Active Natural Products: Agrochemicals* (Vol. 1, pp. 65–94). CRC Press.
- Nene, Y. L., &Thapliyal, P. N. (2000). *Fungicides in Plant Disease Control*. Oxford & IBH Publishing Co.
- Pandey, A. K., Srivastava, M., &Verma, R. K. (2018). Bioherbicidal potential of fungal metabolites against invasive weeds. *Journal of Environmental Biology*, 39(2), 159–165.
- Pitt, J. I., & Hocking, A. D. (2009). *Fungi and Food Spoilage* (3rd ed.). Springer.
- Prasad, S., &Yadav, S. (2018). Mycoherbicides: an eco-friendly approach for weed control. *Journal of Pharmacognosy and Phytochemistry*, 7(1), 1697–1701.
- Varejão, E. V. V., Demuner, A. J., Barbosa, L. C. A., &Barreto, R. W. (2013). Natural products from plant-associated fungi: a new opportunity for the herbicide market? *Biotechnology Advances*, 31(8), 1443–1452.
- Waller, J. M., Lenné, J. M., & Waller, S. J. (2008). *Plant Pathologist's Pocketbook* (3rd ed.). CAB International.
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols* (pp. 315–322). Academic Press.